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ERRATA AND AUTHORS' EMENDATIONS

Page 99, Table 1, in heading "arid" should be "acid."

Page 139, lines 4 and 5, insert "do not" between the words "fibers" and "differ."

Page 140, Footnote 5 should be "PETTENKOFFER, M. J. VON. THE RELATION OF THE AIR TO THE CLOTHES WE WEAR, THE HOUSE WE LIVE IN, AND THE SOIL WE DWELL ON. THREE POPULAR LECTURES DELIVERED BEFORE THE ALBERT SOCIETY AT DRESDEN. Abridged and translated by Augustus Hess. 94 p. London, 1873."

Page 142, third line from bottom, "4.12" should be "6.12;" second line from bottom, "10.12" should be "12.12", "20.12" should be "22.12", "40.12" should be "42.12", and "160.12" should be "162.12."

Page 183, line 17, "labrium" should be "labrum."

Page 184, Figure 2, fourth line in legend, "190" should be "90."

Page 188, Figure 3, fourth line in legend, "585" should be "385."

Page 189, fifth line from bottom, insert "the drop" between the words "in" and "one"; fourth line from bottom, delete "the drop."

Page 197, fourteenth line from bottom, "assuming" should be "assuring."

Page 199, Figure 1, in legend, the formula should be "$y=-0.0207+0.9105x-3.9490\ log.x$."

Page 200, line 6, "$P=0.01$" should be "$P<0.01$."

Page 201, line 17, "the second" should be "other."

Page 207, footnote a, "same" should be "serial."

Page 255, fifteenth line from bottom, insert "sandy" between the words "in" and "soil"; thirteenth line from bottom, insert "the" between the words "between" and "soil," add "s" to "moisture", and insert "tested" between the words "moistures" and "longevity."

Page 288, lines 9, 24, 28, and 30, "she" should be "he."

Page 367, Table 1, it should be indicated that the species *Microbracon gelechiae* (Ashm.) was bred from *L. molesta* by the writer.

Page 448, nineteenth line from bottom, "*n. vorax*" should be "*n. ferax*."

Page 450, nineteenth line from bottom, "*brachyurus* De Man (4), 1884" should be "*brachyuris* deMan (4) 1880."

Page 480, footnote 5, page 482, should be on page 480. It refers to "tuber-line" in line 17.

Page 483, Table 2, column 1, *b* should be omitted after "(A)^b;" column 2, "^{b}1.6" should be "^{b}1.6."

Page 496, Figure 4, first line in legend, "recovery" should be "production."

Page 497, Figure 5, first line in legend, "recovery" should be "production."

Page 513, sixth line from bottom, "inoculation" should be "isolation."

Page 567, Table 3, forty-seventh scientific name, "Viola conadensis" should be "Viola canadensis."

Page 578, line 7, "inhabits" should be "inhibits."

Page 691, fourth line from bottom, insert comma after "that" and delete comma after "probably."

Page 774, sixth line from bottom, "the exceptions" should be "four exceptions."

Page 778, line 21, "$r=0.10$ to 0.50" should be "$r=0.10$ to 0.80".

Page 779, line 2, "$r=_{SO_4}\Delta-r_{SO_4}K$" should be "$r_{SO_4}\Delta-r_{SO_4K}$".

Page 781, line 13, "$r_{RSO_4}>r_R\Delta$" should be "$r_{RC^1}>r_R\Delta$".

Page 781, line 18, delete subsubscript 4 in "$r_R\Delta_4$".

Page 821, line 2, "organic" should be "inorganic."

Page 850, line 21, after the word "important" add the following, "since plants take the disease from infected soil and."

Page 874, Table 2, under "Name," "McCullock" should be "McCulloch."

Page 879, Figure 7, third line of legend, and third line from bottom of page, "McCullock" should be "McCulloch."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 41 WASHINGTON, D. C., JULY 1, 1930 NO. 1

EFFECT OF ENVIRONMENTAL FACTORS UPON THE RESISTANCE OF CABBAGE TO YELLOWS[1]

By J. C. WALKER, *Agent, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and *Professor of Plant Pathology, University of Wisconsin,* and ROSE SMITH, *Research Associate in Plant Pathology, University of Wisconsin*

INTRODUCTION

In a previous paper (*8*)[2] the senior writer described the genetic behavior of resistance in cabbage to the yellows organism, *Fusarium conglutinans* Wr. It was shown that resistance and susceptibility are controlled by a single pair of genes (*Rr*), so far as could be determined from the reaction of the various progenies tested under field conditions in southern Wisconsin on soil naturally infested with the yellows organism.

Several years' work has yielded a number of strains of cabbage which are more constantly and uniformly resistant than any of the mass-selected varieties available to previous workers (*2*, *4*, *8*, *9*). It was the purpose of the present investigation to study under controlled conditions their reaction to environmental factors, primarily temperature. Such inquiry is important for several reasons. From the practical point of view it is desirable to know to what extremes improved strains will maintain their resistance. The distinction between environmental and hereditary factors in the interpretation of the minor variations in severity of disease in susceptible plants is essential as a guide to further selection. A better understanding of this distinction was also germane to studies of the inherent differences in the relation of the parasite to resistant and susceptible host plants.

Considerable work has been previously done upon the relation of temperature to the development of yellows by Gilman (*1*), Tisdale (*7*), and Tims (*6*). Since these studies have been adequately summarized by Jones and associates (*3*), it is unnecessary to discuss them in detail here.

METHODS OF EXPERIMENTATION

The naturally infested soil employed in these studies was taken directly from a thoroughly diseased field in southeastern Wisconsin used over a period of several years for testing the resistance of cabbage strains. For artificial inoculation of soil a single-spore isolation of the organism secured from a diseased plant collected in southeastern Wisconsin was grown for several weeks upon a mixture of sand and corn meal. The culture was then pulverized and mixed thoroughly with soil which had been previously sterilized at a pressure of 15 pounds for several hours. The inoculated soil was used in some experiments during the several weeks immediately following its preparation, and it was used again in other trials after storage for several months.

[1] Received for publication Aug. 20, 1929; issued June, 1930. This study has been supported jointly by the Department of Plant Pathology, University of Wisconsin, with the aid of a special grant from the general research fund of the university, and by the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 15.

Soil-temperature studies were carried out in the Wisconsin soil-temperature tanks described elsewhere (*3*). Plants were grown in infested soil in galvanized-iron cans which were inserted in tanks held with a reasonable degree of constancy at the desired temperatures. The seedlings were grown in soil free from the yellows organism and transplanted to the cans at the desired time. The cans were then kept at 15° C. or lower until the plants had recovered from transplanting and were then removed to the tanks. In records of these experiments the duration was calculated from the time the cans were placed in the tank, care having been taken to hold the soil temperature previous to that time to a point below 15°, the minimum for disease development. Unless otherwise noted, a common air temperature which fluctuated between 15° and 17° was maintained, except on sunny days, when it rose a few degrees higher.

In the study of air-temperature influences along with soil-temperature effects tanks were used which stood in greenhouses in which the air temperatures were regulated at different levels. Although the temperature of the air was not so constant as that of the soil, it was sufficiently constant to give reasonable contrast between the various levels used.

As indicated in the later discussion of experimental results, the typical appearance of yellows in the leaves was used as the chief criterion in recording the appearance and progress of the disease. In certain cases, however, this was insufficient, because of variation in symptoms under different environments. This was especially true at the higher soil temperatures in the case of resistant plants, where stunting of the plants and unnatural mottling of the leaves, not typical symptoms of the disease, were common as a result of exposure to the extreme environment. In these investigations the influence of soil moisture was not studied. For each series, however, the soil moisture was kept uniform throughout by frequent weighings and the addition of sufficient water to maintain the original weight of all pots. Following the evidence from Tisdale's work (*7*) that moderately dry soil gave the greatest quantity of disease, the moisture content was kept at about 60 per cent of the water-holding capacity.

SOURCE OF HOST MATERIALS

As has already been explained in a previous paper (*8*), most commercial varieties of cabbage are extremely susceptible to yellows. In each variety, however, there are ordinarily a few plants that are resistant under field conditions. These are in practically all cases heterozygotes so far as resistance is concerned, and, resistance being dominant, they withstand the attack of the yellows organism. By pure-line selection from such individuals, lines were secured which are homozygous for resistance under field conditions, in Wisconsin at least and where tested in other localities. In like manner individuals selected from commercial varieties growing on noninfested soil in most instances yield progenies that are practically 100 per cent susceptible. Such lines are herein regarded as homozygous for the susceptible character.

A third type of material used in this connection is that referred to as mass-selected resistant. Examples of this are Wisconsin All Seasons and Wisconsin Hollander, resistant varieties now in general commercial use. These differ from the homozygous-resistant lines

in that they were derived from commercial types, not by pure-line selection but by mass selection from the surviving plants on infested soil. They have thus remained to some degree heterozygous for resistance and in each generation continue to produce some purely susceptible individuals. As was pointed out earlier, resistant strains of this type were the only ones available to Tisdale (*7*) and to Tims (*6*). For purposes of more direct comparison the same varieties they used, Wisconsin Hollander and Wisconsin All Seasons, were included in these studies.

Thus the following two resistant and two susceptible types of material were used:

(1) The homozygous-resistant lines were progenies of individual plants, or mixtures of such progenies, which were proved to be homozygous by progeny tests in the field. Progenies 40–350s, 40–353s, 40–354s, 40–420s, 40–421s, 40–426s, 40–524s, 40–530s, and 40–535s were secured by self-pollination of individual plants selected from the All Head Early variety. 40–26–F was a mixture of remnants of selfed progenies from the same variety. 30–25–A was from the seed of several homozygous-resistant plants selected from the Copenhagen Market variety and allowed to be cross-pollinated by insects in an isolated location. 20–27–C was a mixture of remnants from selfed progenies of plants selected from the Jersey Wakefield variety. 20–29–A was the second generation from a homozygous resistant individual of the same variety.

(2) The mass-selected resistant varieties were secured from a reliable commercial source of these seeds. Wisconsin Hollander, Wisconsin All Seasons, and All Head Select were used.

(3) Homozygous-susceptible lines were from plants proved by progeny tests in the field to be homozygous for susceptibility. HG–5s was a selfed progeny from an F_1 hybrid plant resulting from a cross between an All Head Early and a Glory of Enkhuizen susceptible individual. HG–27–A was a mixture of sister progenies coming from the same original cross as HG–5s. Reciprocal crosses were made of two F_1 hybrid plants coming from a cross between an All Head Early and a Copenhagen Market susceptible individual; the flowers of these two plants (HC–19 and HC–3) were not emasculated, but a single camel's-hair brush was worked over the blossoms of each plant and the seed of each was saved separately. Seed from plant HC–3 was used and is designated as HC–3 × 19B. C–29–A was a homozygous-susceptible line from the Copenhagen Market Variety.

(4) Two commercial susceptible varieties, Copenhagen Market and Danish Ballhead, were used. Although these were not quite so homogeneous as the homozygous-susceptible lines, they were shown by field tests to contain a very small percentage of resistant individuals.

In addition to the above-mentioned lots, certain F_1 and F_2 progenies from crosses between resistant and susceptible lines were studied. These are described later in the text.

EXPERIMENTAL RESULTS

COMPARATIVE STUDY OF RESISTANT AND SUSCEPTIBLE LINES

Influence of Soil Temperature

The plants used in experiment 1 had grown in noninfested soil for about six weeks before they were transplanted to infested soil. Two homozygous-resistant lines (40–535s and 30–25–A) were compared

with one commercial susceptible variety (Copenhagen Market). Table 1 shows that the severity of the disease in the susceptible variety increased with rise in soil temperature, reaching a maximum at 26° to 30° C. This is in accord with the findings of Tisdale (*7*) and Tims (*6*) for other susceptible varieties. In the case of the resistant strains, however, there was complete absence of disease at 20° and 22°. At 24° no external symptoms appeared, but upon careful examination of the vascular system there were found a few brownish streaks not unlike those present in greater abundance in susceptible plants at this and other temperatures. All attempts to isolate the fungus from such stems at 24° and 26° were unsuccessful. The significance of the browning of the veins in resistant lines at this temperature will be discussed later. In one resistant line (30–25–A) a few plants showed external evidence of disease at 28°, and both strains had a few such cases at 30°. It thus appeared that the homozygous-resistant lines were exhibiting uniform resistance in this case at a constant soil temperature as high as 26°. This was quite different from the results secured by Tisdale (*7*) and Tims (*6*) with the mass-selected resistant varieties, which showed considerable disease at 20° and comparatively little resistance above that point.

TABLE 1.—*Development of yellows in a commercial susceptible cabbage variety and two homozygous-resistant progenies when grown on naturally infested soil at various constant soil temperatures*

Soil temperature (°C.)	Occurrence of yellows in—					
	A susceptible variety [a]		A resistant variety [b]		A resistant variety [c]	
	Plants	Diseased	Plants	Diseased	Plants	Diseased
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
20	10	60	20	0	23	0
22	10	70	24	0	22	0
24	9	89	24	0	23	0
26	9	100	24	0	23	0
28	8	100	24	0	23	17
30	9	100	24	25	23	13

[a] Copenhagen Market.
[b] Selected from All Head Early, 40–535s.
[c] Selected from Copenhagen Market, 30–25–A.

TABLE 2.—*Development of yellows in two homozygous-susceptible cabbage progenies and eight homozygous-resistant progenies when grown on naturally infested soil at various constant soil temperatures*

Description of strain	Strain No.	Occurrence of yellows at soil temperature of—					
		24° C.		26° C.		28° C.	
		Plants	Diseased	Plants	Diseased	Plants	Diseased
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Homozygous-susceptible	HG–5s	10	100	10	100	10	100
	HC–3×19B	7	100	7	100	6	100
Homozygous-resistant: From All Head Early	40–350s	7	0	7	0	7	57
	40–353s	10	0	9	11	10	0
	40–354s	8	0	8	0	8	25
	40–420s	9	0	9	0	9	22
	40–421s	10	0	9	0	9	22
	40–426s	10	0	10	0	10	10
	40–524s	7	0	6	0	7	0
From Jersey Wakefield	20–27–C	7	0	10	20	10	40

In experiment 2 (Table 2) the study was extended to two homozygous-susceptible lines (HG–5s, HC–3 × 19B), a number of homozygous-resistant progenies selected from All Head Early (40–350s, etc.), and a mixture of several homozygous-resistant progenies from Jersey Wakefield (20–27–C). In this instance the plants were much younger when exposed to infection, which exposure took place only 26 days from the time the seed was sown. The experiment was continued for 40 days. Only the critical temperatures 24°, 26°, and 28° C. were used. At all temperatures the susceptible strains succumbed completely. At 24° all the resistant lines remained free from disease symptoms. At 26° two progenies showed slight disease, and at 28° all but two progenies showed more or less disease. Thus in comparison with experiment 1, these lots were as resistant at 24°, but two of them were less stable at 26°, and most of them less so at 28°.

In experiment 3 a homozygous-susceptible line (HG–5s), two homozygous-resistant lines (40–420s and 20–27–C), and three mass-selected resistant varieties were used. (Table 3.) The plants in this case were 32 days old when exposed to the various soil temperatures (18°–33° C.), and the experiment was continued for 33 days. As noted earlier by Tisdale (*7*) and Tims (*6*), the disease developed very slowly at 18°, even in the susceptible strain, while its incidence also was much reduced at 33°. These extremes appear to limit the progress of the parasite materially, and this probably accounts in a large measure for the reduction in the amount of the disease. The behavior of the homozygous-susceptible and the homozygous-resistant lines otherwise coincides in general with that observed in experiments 1 and 2. As noted in the experiments of Tisdale and Tims, a small percentage of plants of Wisconsin Hollander and Wisconsin All Seasons became diseased at 21°, and the percentage increased with the rise in temperature up to 30°. The difference between the homozygous-resistant lines and mass-selected varieties appears at 21° and higher temperatures. It is evident that the latter, though quite resistant under Wisconsin field conditions, are inherently much more susceptible than the homozygous lines, while their exposure to constant temperatures above 21° reveals that they are unsuited to such conditions. The cans from this series were photographed near the close of the experiment, and some of them are shown in Figure 1.

TABLE 3.—*Development of yellows in susceptible and resistant cabbage progenies when grown on naturally infested soil at various constant soil temperatures*

[Ten plants of each strain at each temperature except 33°, where there were 20 plants]

Description of strain or variety	Strain No. or variety	Percentage of plants yellowed when grown at soil temperature of—					
		18° C.	21° C.	24° C.	27° C.	30° C.	33° C.
Homozygous-susceptible	HG–5s	50	100	100	100	100	100
Homozygous-resistant	40–420s	0	0	0	0	40	25
	20–27–C	0	0	0	10	80	35
Mass-selected resistant	Wisconsin Hollander	20	30	50	80	90	25
	Wisconsin All Seasons	0	20	50	20	70	50
	All Head Select	0	20	20	60	80	60

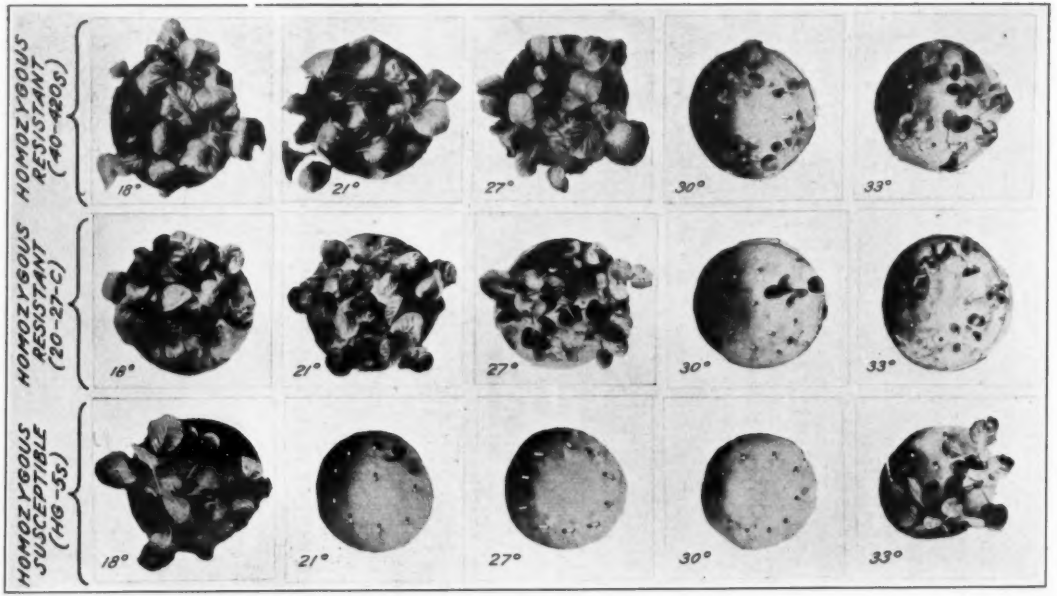

FIGURE 1.—Pots from experiment 3 (Table 3), photographed near the close of the experiment. The white markers indicate where the dead plants were removed. Homozygous-resistant progenies (40-420s and 20-27-C) and a homozygous-susceptible progeny (HG-5s) are shown. All plants of 40-420s remained healthy from 18° to 27° C., whereas at 30° the plants were stunted and some had died. They did not show typical yellows, however. The same is true of 20-27-C, except that one plant died at 27°. Some plants of the susceptible line (HG-5s) were still healthy at 18°, but at 21° and up to 30° all were diseased and all but one had died. At 33°, as in the resistant lines, the disease was less destructive than at 30°. Apparently the activity of the fungus was reduced at this temperature

In the three experiments just described certain new facts are brought out. In the first place, the homozygous-resistant strains, so called because of their perfect resistance under repeated field tests, are extremely resistant at quite high temperatures. Plants of the ages used were practically all stable up to and including constant temperatures of 26° to 27° C. Above this point the plants became stunted, showed a pale yellowing of the leaf parenchyma, and exhibited some browning of the vascular bundles. The external appearances of disease were not typical of those in the susceptible lines at these or lower temperatures. The browning of veins was very rarely accompanied by the presence of the organism.

It was important to determine whether this peculiar reaction of resistant plants at high temperature was due to the attack of the parasite or to the direct effect of the temperature upon the plants. The behavior of plants in infested and in noninfested soil over a range of temperature was therefore studied. (Experiment 4.) In order to make the infested and the noninfested soil as comparable as possible, a quantity of the latter was divided and to one portion inoculum of *Fusarium conglutinans* was added. A series of soil-temperature tanks ranging at 2-degree intervals from 18° to 28° C. were then set up so that in each tank there was one can each of homozygous susceptible plants (C–29–A) and one can each of homozygous resistant plants (20–29–A) in inoculated and in uninoculated soil. The plants were 43 days old at the beginning of the experiment, and the latter was run for 30 days. Six plants were placed in each can. In the inoculated soil the results were similar to those in the naturally infested soil in experiments 1, 2, and 3. The susceptible plants showed typical yellows, the severity increasing with the temperature. The resistant plants remained perfectly healthy up to 24°; they showed some atypical signs at 26°, and the atypical symptoms were severe at 28°. In the uninoculated soil no signs of disease whatever were noted, even at the highest temperature. This fact shows that this rather abrupt appearance of pathological symptoms in the resistant plants at 26° and above is the result of the yellows organism in the soil and not the effect of high temperature alone. The cans from the 24° and the 28° tanks, photographed on the thirteenth day, are shown in Figure 2.

Histological studies subsequently reported (*5*) indicate that there is invasion of the root tips of homozygous-resistant plants with little or no penetration of the vascular system. Furthermore, at 28° and above, the root systems of cabbage are at their low ebb of growth, while the causal organism is most active (*7*). The reduced rate of root growth, with invasion of the absorbing regions of the root system, is probably responsible for the gradual stunting and death of the resistant plants at 28° and above. Moreover, it is to be emphasized that this breaking down of resistance is of quite a different order in external appearance and in internal histology from the normal development of disease in susceptible plants at these or other temperatures.

The distinct difference between commercially successful mass-selected resistant varieties and pure-lined homozygous-resistant lines in their temperature reactions is the second matter of importance to be noted in these results. Many plants of the mass-selected strains behaved like susceptible ones under these controlled conditions, which is in accord with Tisdale's (*7*) and Tims's (*6*) results. While this may

not appear to be in accord with the fact that these varieties are commercially successful upon infested soil, it should be pointed out that it is not uncommon to find a field of these mass-selected varieties with as high as 50 per cent of the plants showing slight symptoms of disease. As has been suggested before (*8*), it is possible that in the selection of these varieties mildly susceptible individuals were not eliminated and as a result they are apparently present in considerable numbers. Under average seasonal conditions where the crop is started during the cooler spring months so that the plants reach considerable size before infection occurs, these mildly susceptible individuals are not easily distinguished. The test under controlled environment is apparently more severe and the differences between them and homozygous-resistant plants are accentuated.

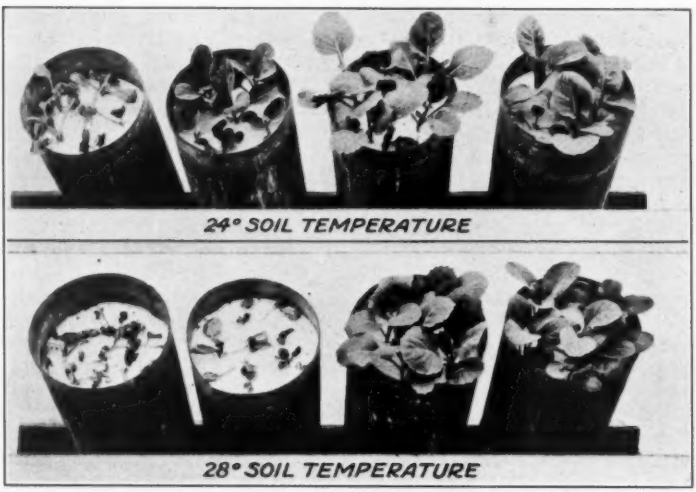

FIGURE 2.—Comparison of resistant and susceptible lines of cabbage upon inoculated and uninoculated soil at 24° and 28° C. Cans in left-to-right order in each row contain (1) susceptible plants in inoculated soil; (2) resistant plants in inoculated soil; (3) susceptible plants in uninoculated soil; (4) resistant plants in uninoculated soil. See further explanation in the text

INOCULATED STERILIZED SOIL

In the first three experiments naturally infested soil was used. While this method yielded results more comparable with those secured under field conditions, it did not afford a picture of the behavior of the plants exposed to the yellows organism when it was removed from competition with other soil organisms and thus allowed to accumulate in greater abundance. In experiment 4 the plants were 50 days old when exposed to various soil temperatures. A commercial susceptible variety, a mass-selected resistant variety, and a homozygous-resistant strain were used. There were four to seven plants in each can. The percentages of diseased and of dead plants were recorded after 30 days. The final results are shown in Table 4.

TABLE 4.—*Development of yellows in a susceptible cabbage variety, a mass-selected resistant variety, and a homozygous-resistant progeny when grown in naturally infested and inoculated sterilized soils at various constant soil temperatures*

[In each individual lot 7 plants were used, except of Wisconsin Hollander at 26°, where only 6 plants were used in naturally infested soil, and at 28°, where 5 and 6 plants were used, respectively, in naturally infested and inoculated sterilized soil]

Soil temperature (° C.)	Occurrence of yellows in susceptible commercial (Copenhagen Market) variety grown in—				Occurrence of yellows in mass-selected resistant (Wisconsin Hollander) variety grown in—				Occurrence of yellows in homozygous-resistant (40–539, selected from All Head Early) variety grown in—			
	Naturally infested soil		Inoculated sterilized soil		Naturally infested soil		Inoculated sterilized soil		Naturally infested soil		Inoculated sterilized soil	
	Diseased plants	Dead	Diseased plants	Dead	Diseased plants	Dead	Diseased plants	Dead	Diseased plants	Dead	Diseased plants	Dead
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
20	86	57	100	100	14	0	43	14	0	0	0	0
22	100	86	100	100	57	14	43	29	0	0	0	0
24	100	100	100	100	100	57	100	86	0	0	0	0
26	100	100	100	100	100	33	100	71	0	0	[a] 100	0
28	100	100	100	100	100	80	100	100	[a] 100	[a] 14	[a] 100	[a] 43
30	100	100	100	100	100	100	100	100	[a] 100	[a] 100	[a] 100	[a] 100

[a] These plants did not show typical symptoms of yellows but were nevertheless stunted and showed yellowish spots on the leaves, dying back of the leaf margins, and more or less browning of the veins. Practically all attempts to isolate the organism from them were unsuccessful. Differences are illustrated in Figure 3.

It is clear that the increased potentiality of the inoculum which results from sterilization of the soil to remove the soil flora, temporarily at least, from competition with the yellows organism has an immediate effect upon the disease. The incubation period is somewhat shortened and the progress of the disease is hastened. Aside from these effects it is quite consistently noticeable that the total percentage of plants diseased in the susceptible and mass-selected resistant groups is increased in the inoculated soil. This is probably the result, in part at least, of a more uniform distribution of the parasite in the soil. The relative differences between susceptible and resistant strains are not changed; but the homozygous-resistant line showed, in inoculated sterilized soil, the pathological response characteristic of this type at high temperatures at a slightly lower point than in previous experiments wherein naturally infested soil was used.

INFLUENCE OF AIR TEMPERATURE

In the first five experiments the air temperature during most of the time was at 15° to 17° C., although for a few hours during sunny days it rose a few degrees higher. This is somewhat lower than might often obtain under natural conditions during midsummer. Consideration was next given to the influence of the air temperature as well as of the soil temperature upon the expression of resistance.

In experiment 6 a homozygous-susceptible line (HG–27–A) and a homozygous-resistant line (40–26–F) were included. Plants 45 days old of each were used, and the experiment was run for 27 days in naturally infested soil. Soil temperatures of 20° and 24° were maintained at air temperatures of 15° to 17° and of 28°, respectively. In the susceptible variety the disease developed most rapidly at the 28°

air temperature in the two respective soil temperatures, which is in accord with the earlier findings of Tims (*6*). The final results (Table 5) show, however, that even at this air temperature the homozygous-resistant line remained healthy.

TABLE 5.—*Development of yellows in susceptible and resistant lines of cabbage at various air and soil temperatures*

Temperature of—		Occurrence of yellows in—			
		Susceptible line		Resistant line	
Air	Soil	Plants	Diseased	Plants	Diseased
° *C.*	° *C.*	*Number*	*Per cent*	*Number*	*Per cent*
15	20	5	100	10	0
	24	5	100	10	0
28	20	5	100	10	0
	24	5	100	10	0

EFFECT OF WOUNDING THE ROOT ON INFECTION AND RESISTANCE

It became of interest to determine whether or not severe pruning of the root system would permit the development of disease in resistant plants. Plants 10 weeks old of a homozygous-resistant strain (40–535s) and a commercial susceptible variety (Copenhagen Market) were transferred to a bench of naturally infested soil, a portion of each strain being handled as follows: (1) Transplanted with the least possible injury to the roots; (2) roots pruned moderately before resetting; and (3) all lateral roots pruned close to the taproot. The disease was recorded for each lot as it appeared, and the data are summarized in Table 6. It is significant that even the severest degree of root pruning did not bring about infection of the resistant strain. In the susceptible strain the appearance of the disease in slightly pruned plants was delayed several days as compared with the moderately and severely pruned plants, but at the end of the period the total infections in the three lots were not significantly different.

TABLE 6.—*Effect of varying degrees of root pruning at transplanting upon the occurrence of yellows in a resistant and a susceptible strain of cabbage*

Strain or variety	Degree of root pruning	Total number of plants	Number of yellowed plants at end of—							
			17th day	19th day	23d day	25th day	30th day	33d day	37th day	44th day
40–535s (resistant)	Slight	19	0	0	0	0	0	0	0	0
	Moderate	20	0	0	0	0	0	0	0	0
	Severe	20	0	0	0	0	0	0	0	0
Copenhagen Market (susceptible)	Slight	10	0	0	4	4	7	9	9	9
	Moderate	16	3	4	6	7	7	13	13	13
	Severe	17	2	4	4	5	7	12	14	15

GROWING CONDITIONS BEFORE EXPOSURE TO THE PARASITE IN RELATION TO SUSCEPTIBILITY AND RESISTANCE

Throughout the experiments just reported plants for transplanting were commonly grown at comparatively low temperatures—about 15° C.—and were then transferred to infested soil and placed at various

soil and air temperatures. The question arose as to whether the conditions under which a plant is grown before exposure to the parasite under a given environment have any important bearing upon susceptibility or resistance. In fact Tims (*6*) suggested that plants grown at a comparatively high temperature became diseased less readily than those grown at lower temperatures more favorable for growth of the host. An experiment was therefore planned to yield some evidence on this point.

Seeds of a homozygous-resistant line (20–27–C) and of a homozygous-susceptible line (HC–27–A) were sown in cans of noninfested soil. They were placed in one set of soil-temperature tanks running at 20°, 24°, 28°, and 32°, at each of two air temperatures, 15°–17° and 28°. Thus each of these two varieties was growing under eight different combinations of air and soil temperature. After 32 days they were transplanted to naturally infested soil. After standing 3 days at about 20° for the plants to recover from transplanting, the cans were so divided that one half of the plants from each combination of temperatures was kept at a soil temperature of 24° and an air temperature of 28°, while the other half was held at 24° soil and 15°–17° air. The plants were thus held for 23 days. By that time every plant in the susceptible line was diseased and most of them were dead. No disease appeared in any of the resistant lines throughout the series. The rate of appearance of the disease and death of the susceptible plants, as shown in Table 7, does not indicate any marked predisposing influence of the growing conditions previous to infection upon disease development in plants of this age. It appears that the method employed of producing the plants at temperatures favorable to growth and later shifting them rather suddenly to various temperatures for exposure to the parasite does not introduce a significant experimental error.

TABLE 7.—*Relative development of yellows in susceptible cabbage plants[a] grown for about five weeks in noninfested soil at various soil and air temperatures and then transplanted to infested soil and exposed to a soil temperature of 24° and air temperatures of 15°–17° and 28° C.*

Air temperature after transplanting (°C.)	Temperatures before transplanting from clean soil		Total number of plants	Number of yellowed plants at the end of—				Number of dead plants at the end of—						
	Air	Soil		10 days	12 days	14 days	16 days	12 days	14 days	16 days	18 days	19 days	22 days	23 days
	°C.	°C.												
15–17	15–17	20	5	2	4	5	------	0	0	0	0	1	3	3
		24	5	0	4	5	------	0	0	0	0	3	5	------
		28	5	4	4	5	------	0	2	3	4	4	5	------
		32	5	1	4	5	------	0	0	2	2	2	4	4
	28	20	5	0	5	5	------	0	0	0	3	3	5	------
		24	5	0	0	1	5	0	0	0	0	0	3	3
		28	5	0	1	3	5	0	0	1	1	3	4	5
		32	4	2	3	4	------	0	1	1	1	3	4	------
28	15–17	20	5	4	4	5	------	0	0	2	3	3	4	4
		24	5	0	5	5	------	0	0	3	4	4	4	4
		28	4	0	3	3	4	1	1	3	4	------	------	------
		32	4	0	2	3	4	0	0	1	2	3	3	4
	28	20	5	2	4	4	5	0	3	3	4	4	5	------
		24	5	0	3	3	5	0	3	3	3	3	4	4
		28	4	3	3	4	------	0	2	2	4	------	------	------
		32	4	1	2	4	------	0	1	1	4	------	------	------

[a] HC–27–A.

STUDY OF REACTION OF RESISTANT-SUSCEPTIBLE HYBRIDS

In the light of the evidence brought together in the foregoing pages, it is of interest to consider the reaction of resistant-susceptible hybrids as reported earlier (*8*).

F_1 Hybrids

The F_1 hybrids from resistant and susceptible parents were first studied. Several progenies were grown in both inoculated sterilized and naturally infested soil at various temperatures. The hybrids, which were fully resistant in field tests, were found to be quite as resistant as homozygous-resistant plants except for the fact that in the case of four progenies they showed stunting and high-temperature symptoms at slightly lower temperatures. (Table 8.) In a fifth case (20–27–D) no pathological effects were noted at 26° C. in naturally infested soil. Certain of the lots are shown in Figure 3.

TABLE 8.—*Development of yellows in F_1 cabbage hybrids from resistant-susceptible crosses, and commercial susceptible and homozygous-resistant lines*

Age of plant	Duration of experiment	Description of strain or variety	Strain No. or variety	Occurrence of yellows in plants grown on—							
				Naturally infested soil at—				Inoculated sterilized soil at—			
				24° C.		26° C.		24° C.		26° C.	
				Plants	Diseased	Plants	Diseased	Plants	Diseased	Plants	Diseased
Days	*Days*			*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
50	30	Susceptible	Copenhagen Market	7	100	7	100	7	100	7	100
		Resistant	40-530s	7	0	7	0	7	0	7	* 100
		F_1 hybrids	H-2×5-21	7	0	4	* 100	4	* 100	4	* 100
82	28	Susceptible	Copenhagen Market	4	100	4	100	4	100	4	100
		Resistant	40-530s	4	0	4	0	4	0	4	* 100
		F_1 hybrids	5-32×C-1	4	0	4	* 100				
		do	40-335×H-35	4	0	4	* 100	4	* 75	4	* 100
		do	5-32×H-35	4	0	4	* 100	4	* 100	4	* 100
40	32	do	20-27-D	10	0	10	0				

* The symptoms in these cases were not those of typical yellows that occurred in the susceptible strain but were the atypical signs current in resistant strains at high soil temperature as described elsewhere in the text.

F_2 Hybrids

From the evidence already presented it was to be expected that F_2 hybrid progenies would react according to the constitution of their segregants. Approximately 25 per cent, being homozygous-resistant, should survive as transplanted seedlings up to 26° C. Approximately 50 per cent, being heterozygotes, should survive at 24° and some become stunted at higher temperatures in naturally infested soil. The remaining one-fourth, being homozygous-susceptible, should succumb at 22° or above. Variation in the progress of the disease in the susceptible plants should also result, depending upon soil temperature.

The experiment reported in detail was conducted with a single F_2 progeny (5HIA) which in field trials showed 28.6 per cent diseased plants in 1926 and 22.2 per cent in 1927. Plants 37 days old were exposed to a range of soil temperatures in naturally infested soil. At

18° and 20° somewhat fewer than the expected number were affected; at 22° exactly one-fourth were diseased. (Table 9.) At 24° slightly above 25 per cent were diseased. At 26° the percentage was significantly higher, while at 28° only about one-fourth survived. At

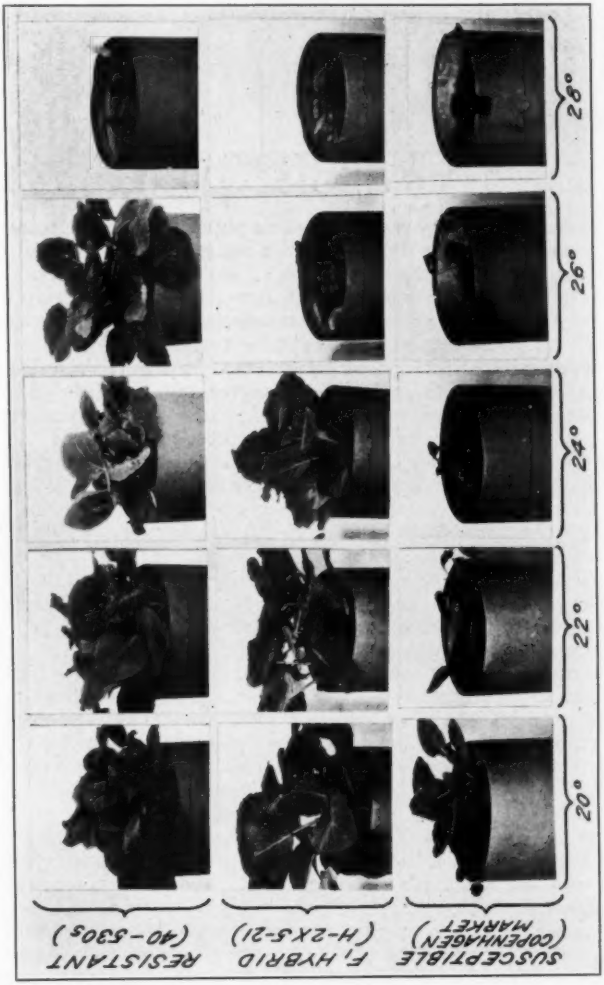

FIGURE 3.—Comparison of development of yellows in a homozygous-resistant progeny (40-530s), a susceptible variety (Copenhagen Market), and a hybrid progeny from a cross between a resistant and a susceptible plant (H-2XS-21). Plants grown on infested soil at various soil temperatures. (For details see Table 8.) The homozygous-resistant strain was healthy at 26° C. but decidedly stunted at 28°, whereas the hybrid plants were healthy at 24° but stunted at 26°. One plant of the susceptible strain was still healthy at 20°, but all plants were diseased or dead at 22° and above

26° part of the plants and at 28° all of those which survived showed distinct stunting of the type observed in heterozygous and homozygous-resistant plants at these temperatures. No attempt was made to differentiate the two types when the counts were recorded.

TABLE 9.—*Development of yellows in an F_2 hybrid progeny from a cross between a homozygous-susceptible and a homozygous-resistant parent*

Soil temperature	Total number of plants	Yellowed plants	
°C.		*Number*	*Per cent*
18	58	12	20.6
20	59	12	20.3
22	60	15	25.0
24	58	16	27.5
26	60	19	31.6
28	60	44	73.3

SUMMARY

The purpose of the study reported in this paper was to determine the effect of environmental factors upon the resistance or susceptibility in various strains of cabbage to yellows (*Fusarium conglutinans*).

The hosts tested consisted of homozygous-resistant progenies, homozygous-susceptible progenies, mass-selected resistant varieties in commercial use, and commercial susceptible varieties.

In the susceptible strains and mass-selected resistant varieties the typical disease symptoms appeared in increasing percentages with increase in soil temperature to about 28° C., and were retarded somewhat at 33°. In homozygous-resistant strains no evidence of disease was found up to 24° in naturally infested soil when young transplants were used. Slight evidence of disease was found in a few cases at 26° and to a greater degree at still higher temperatures. At this higher range the symptoms were not typical of yellows, and the fungus was rarely isolated from such plants. The homozygous-resistant lines, therefore, reacted in a distinctly different manner from the susceptible and mass-selected resistant types.

The increase of inoculum secured by steam sterilization of the soil and reinoculation with a pure culture of the yellows organism resulted in more uniform infection and more rapid disease development in susceptible and mass-selected resistant types, but had no effect upon the homozygous-resistant lines, except that the atypical high-temperature symptoms were evident at a slightly lower temperature.

Increase in air temperature up to 28° C. hastened the development of disease but did not alter the distinct difference in reaction between homozygous-resistant lines and the other types used.

Severe pruning of the root system during the process of transplanting served to shorten the incubation period in susceptible plants, but it did not facilitate the production of yellows in homozygous-resistant plants.

The temperature at which plants were grown prior to exposure to the parasite did not appear to affect the rate of disease development, nor did it have any influence upon the stability of resistance in homozygous lines.

Heterozygous plants, i. e., those resulting from crosses between resistant and susceptible parents, reacted as homozygous resistant except that they usually showed atypical high-temperature symptoms at a slightly lower tenperature.

At soil temperatures around 22°–24° C. transplants of F_2 segregating progenies from resistant-susceptible crosses showed approxi-

mately 25 per cent typical susceptible plants, as they had previously shown in the field. As the temperature increased above this point the atypical high-temperature symptoms appeared, as was expected, in the heterozygous and homozygous resistant members of the population. As the temperature fell below this point the full expression of typical disease symptoms in homozygous-susceptible plants decreased.

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A CYTOLOGICAL STUDY OF CABBAGE PLANTS IN STRAINS SUSCEPTIBLE OR RESISTANT TO YELLOWS [1]

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INTRODUCTION

Yellows of cabbage (*Fusarium conglutinans* Wr.) and the progress in its control through the development of resistant varieties have been discussed elsewhere (*7, 8, 9, 16*).[3] Out of this breeding work certain pure lines of cabbages have been secured (*14*), some of which are completely resistant and others completely susceptible under field conditions. In genetic studies with this material resistance has been shown to behave as a single dominant Mendelian factor.

When dependable homozygous-resistant and susceptible lines became available the writers began a study to determine if possible the cause of this marked resistance to the yellows organism possessed by certain lines of cabbage and not by others. The problem was approached from two angles: (1) The influence of a number of environmental factors upon the stability of resistance was studied. The results are presented in the paper immediately preceding this one (*15*). (2) A cytological study of infection and the relation of host to parasite in resistant as compared with susceptible lines was undertaken. The purpose of this study was to find the answers to several questions. Does the yellows organism invade the roots of resistant plants? If so, how extensively are the roots attacked? Are there any visible morphological differences between resistant and susceptible roots? Are there any visible differences in the reaction of the two types of host to the invading parasite?

MATERIALS AND METHODS

HOST MATERIALS

As pointed out in a previous paper (*14*), most of the commercial varieties of cabbage, with the exception of those recently developed for resistance to yellows, contain a very large percentage of susceptible plants and practically always a small percentage of resistant ones. From such commercial strains were made selections which appeared to be homozygous for susceptibility under field conditions. In the course of this cytological work both commercial and selected materials were used. During the early part of the investigation, particularly when selected susceptible material was not yet available, a

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[2] The writers wish to acknowledge with thanks the advice and suggestions of Miss Helen Johann and Prof. L. R. Jones during the progress of this investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 34.

strain of the Copenhagen Market variety listed as Vaughan's Reselected Copenhagen, and designated in this paper as VRC, was used. In tests both in the field and in the greenhouse under controlled environmental conditions this strain was found to be very susceptible. Later, seed was used from a cross (C–16 × 31B) between two Copenhagen Market plants, a progeny which had been shown to be homozygous for susceptibility by the fact that it succumbed very promptly to the disease under field and greenhouse conditions. A third lot, equal to the latter in susceptibility, was a mixture of seeds from tested homozygous-susceptible plants coming from a cross between the two varieties All Head Early and Glory of Enkhuizen. This is referred to as HG–27A.

For the study of the resistant host several progenies from all Head Early were used. These were secured by self-pollination of individual plants from a line that had been selected for several years. The progenies used in this work were tested on infected soil in the field and in the greenhouse and showed no signs of disease except for certain abnormal symptoms when grown at a constant soil temperature of 26° C. or above. The progeny numbers were 40–350s, 40–353s, 40–354s, 40–420s, 40–421s, 40–426s, and 40–524s. The behavior of these and other progenies under varying conditions is discussed in another paper (*15, Table 2*). They represent what has been referred to as "homozygous-resistant" plants so far as resistance to the yellows organism is concerned (*14*).

SOURCE OF INOCULUM

The culture of *Fusarium conglutinans* used was isolated in November, 1926, from diseased cabbage grown at Racine, Wis. From a single-spore culture the inoculum was increased on potato-dextrose agar. Isolations of the fungus from Fayetteville, Ark., Crystal Springs, Miss., and Racine, Wis., respectively, were also employed for comparative purposes.[4]

METHODS OF STUDYING RELATION OF FUNGUS TO HOST

Fungal penetration was studied in young seedlings grown on agar. The seeds were surface sterilized by immersing them in a 1–1,000 solution of corrosive sublimate for five minutes. After being thoroughly washed in several changes of sterile distilled water they were placed in Petri dishes of soil-extract agar. Twenty-four to forty-eight hours later the uncontaminated seedlings were transferred to other Petri dishes of soil-extract agar and arranged in a row, about equidistant from one another, with the roots all pointing in the same direction. Inoculum was applied at definite points—in front of the rootcap, along the sides of the root, and in the vicinity of root hairs. The Petri dishes, arranged in wire baskets so that the seedlings were held in a normal position, were placed in a constant-temperature chamber kept at 24° C.

Studies of the distribution of the fungus in the host were made with older plants grown in soil. Surface-sterilized seeds were sown in a cool greenhouse in flats of sterilized soil. When 30 to 45 days old the seedlings were transplanted to cans of artificially inoculated

[4] These last 3 cultures were provided by L. M. Blank.

soil. This was prepared by autoclaving field soil at a pressure of 10 pounds for three hours on two alternate days. Twenty-four hours later the soil was inoculated uniformly with the fungus, which had been grown in pure culture on a mixture of corn meal and sand. After transplantation of the seedlings the cans were placed in tanks in which the desired constant soil temperatures were maintained.

Considerable difficulty was experienced in removing young growing roots from the soil with their parts intact. The best results were secured by taking up a plant with a large clump of soil and gently agitating it in a vessel of water until it was freed of soil particles. After being surface sterilized, thin slices of the sections being fixed were plated out on potato-dextrose agar. Those sections which yielded *Fusarium conglutinans* and no other fungus were dehydrated and infiltrated with paraffin.

Medium chrom acetic, Gilson's, and formol acetic alcohol fixatives were employed. For detailed study of the fungus in the host Gilson's fixative gave the most satisfactory results. The sections were cut from 10μ to 13μ in thickness. Flemming's triple and Delafield's haematoxylin and safranine were the stains used.

ANATOMY OF THE NORMAL ROOT

For the sake of clarity in the account that follows, a brief description of the anatomy of the young healthy cabbage root is given. As no morphological differences were observed in the resistant and susceptible strains studied, they are not discussed separately.

The growing tip of the young root is protected by a thin cap of cells. Slightly back of it the outermost layer of the primary meristem, the forerunner of the epidermis, differentiates. Its cells are radially elongated and rich in protoplasm. Tangential division of its cells near the apex renews the rootcap from within as its outermost parts are lost. (Fig. 1.) Posteriorly, the cells become elongated axially and the outside wall may be pushed outward to form root hairs.

The primary cortex is made up of four or five rows of elongated cylindrical cells separated from one another by large intercellular spaces. The layer surrounding the endodermis shows peculiar thickenings within 2 mm. of the growing point. (Fig. 2, D.) Both its radial and cross walls become heavily suberized (fig. 2, A) and form a 4-cornered frame which is firmly wedged in the cell and serves to a certain degree as support. On the inner tangential face of these cells, i. e., the wall facing the endodermis, a netlike mesh is developed. (Fig. 2, B.) Occasionally an incomplete net is formed on the opposite wall. This heavily suberized cortical layer completely surrounding the endodermis forms an important protective sheath (*17*).

Both this layer and the endodermis differentiate at about the same time. The radial thickenings of the former are much more pronounced than those of the latter. The identity of the endodermis is determined by its location, immediately outside the pericycle, rather than by the small inconspicuous strips radially placed. The cortex extends posteriorly for a distance of 2 to 3 cm. Its cells enlarge somewhat as the root maturates. The outer vacuolated ones soon collapse and pull apart (fig. 2, A); the inner ones are stretched and torn, and gradually the whole tissue is detached.

The pericycle of the stele is a single-layered concentric ring next to the endodermis. Its rectangular cells are small in cross section. (Fig. 2, C.) From meristems arising in it apparently opposite the xylem points the lateral roots originate. As these cells increase in number and size the walls of the endodermal and cortical cells are pushed apart. Their suberized thickenings form a protective coat about the developing rootlet.

Differentiation of the primary vascular tissue begins 2 to 3 mm. behind the growing region next to the pericycle. The sieve tubes of the protophloem are the first to differentiate. (Fig. 1.) Alternating with the two primary phloem groups, the elements of the two primary

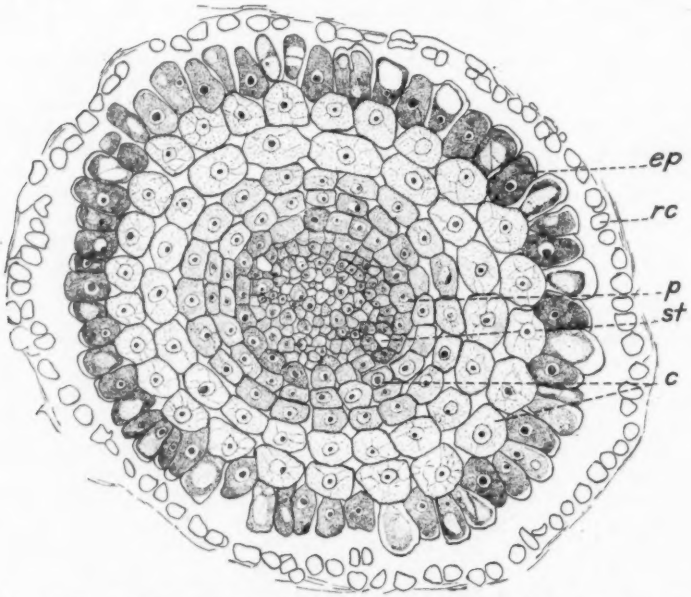


FIGURE 1.—Cross section through the primary meristem of a young cabbage seedling root: *rc*, Rootcap; *ep*, forerunner of epidermis; *c*, forerunner of primary cortex, four and five layers deep; *p*, pericycle; *st*, sieve tube. The primary phloem, but not the primary xylem, has begun to differentiate. × about 200

xylem groups next become distinguishable. (Fig. 2, C.) The protoxylem groups progress centripetally (fig. 2, D and A) until they meet to form the xylem plate. From then on increase in the primary xylem takes place to the right and left of the plate.

Secondary growth is initiated when the seedling is about 3 weeks old, growing at a soil temperature of about 24° C. under greenhouse conditions. Bands of meristem arising between the primary xylem and phloem and gradually extending completely around the xylem cut off secondary tissues. Simultaneously with the increase in the diameter of the stele the cortex is slowly loosened and finally sloughed off. Tangential division of the pericycle gives rise to a secondary protective tissue, the periderm.

EFFECT OF SOIL STERILIZATION ON THE ROOTS OF CABBAGE

The development of the fungus subsequent to penetration was studied in plants grown upon infested soil. In order to eliminate as

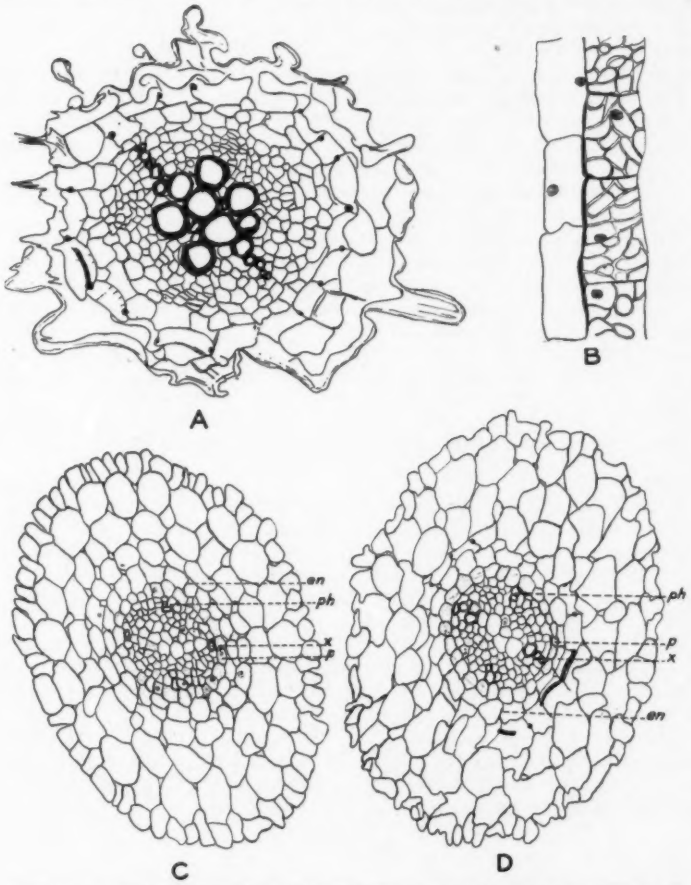


FIGURE 2.—A, Cross section of a young cabbage-seedling root in the stage where the epidermis and the outer cortex are collapsed. The cortical layer surrounding the endodermis shows its characteristic thickenings in both cross and radial walls. × 173. B, Tangential longitudinal section of the cortical layer surrounding the endodermis. Note the characteristic net on the wall facing the endodermis. The section passes through the suberized thickening of one of the radial walls. × 473. C, Cross section through the zone of maturation in a young root. *en*, endodermis; *p*, pericycle; *ph*, phloem; *x*, xylem. The cells of the epidermis and cortex are vacuolate, the radial walls of endodermis are slightly suberized, and the primary xylem points have been initiated. × 173. D, Cross section of slightly older region of the same root. Some cells of the cortical layer surrounding the endodermis have suberized cross and radial walls. The abbreviations have the same meaning as in C. × 173

far as possible the presence of other soil fungi, sterilized soil inoculated with the causal organism was used. This introduced the complication of injury to the root system due to toxins released by heating the soil. In order to understand the nature of this injury a study was made of

the roots of plants (C–16 × 31B) which had grown from seed for one month upon recently sterilized soil and upon unsterilized soil.

The roots of seedlings grown in freshly sterilized soil and examined three weeks to a month later exhibited different degrees of injury. Many of the very young ones were more or less discolored, varying from a dirty-white or yellow to a yellow-brown gum color; the older roots were less yellowed. The cortex of deeply yellowed roots was somewhat collapsed, and occasionally the growing region had disintegrated. Microscopic examination of the fixed sections of these same roots often showed that the epidermis had disappeared and that the walls of the outer cortex were thickened. In some instances a few of the vessels were filled with a gumlike substance. Young roots slightly discolored and older roots showing little or no discoloration seldom showed this response.

The roots of seedlings grown on unsterilized soil were decidedly white and seldom exhibited any discoloration whatsoever. A microscopic examination of young roots showed that the walls of the epidermis and cortex were normal in thickness and that there was a total absence of plugged vessels.

In the light of this evidence it became important not to confuse the effects of soil toxins with the possible effects of the invading fungus upon the host cells.

RELATION OF FUSARIUM CONGLUTINANS TO SUSCEPTIBLE STRAINS OF CABBAGE

Very little histological work has been reported on the relation of *Fusarium conglutinans* to its host. Gilman (*5*) isolated small amounts of the fungus from the stems of cabbage after their leaves had become markedly yellowed. He found the hyphae and conidia confined to the xylem in the living host but after its death traversing all tissues and sporulating both within and at the surface. He believed that it attacked the roots first. Tisdale (*13*) observed the hyphae entering the root hairs of cabbage seedlings grown on artificial media. Both these workers presumably used commercial susceptible varieties, but they made no definite statement in regard to this point.

The results of the present study will be discussed (1) in relation to penetration by the fungus and (2) in relation to its subsequent development in the susceptible host. The drawings, unless otherwise specified, are from preparations of VRC.

PENETRATION

Studies of fungal penetration were made with seedlings grown at a temperature of 24° C. on inoculated soil-extract agar in the manner previously described. The plants were examined at 24-hour intervals, and the seedlings were fixed one to eight days after inoculation. Microscopic examination of the plates 48 hours after inoculation showed the fungus growing plentifully on the agar. Many of the hyphae wove themselves around the root hairs, some were in contact with the roots, and still others were seen penetrating the embryonic tissues.

More than 200 roots inoculated in the region of the root hairs were fixed and examined. In no instance was the fungus observed within a root hair filled with protoplasmic contents. Occasionally it was

seen in older hairs more or less devoid of contents and somewhat shriveled. (Fig. 3, A. D.) Penetration by way of the root hairs in the roots examined was so infrequent that it was of no consequence

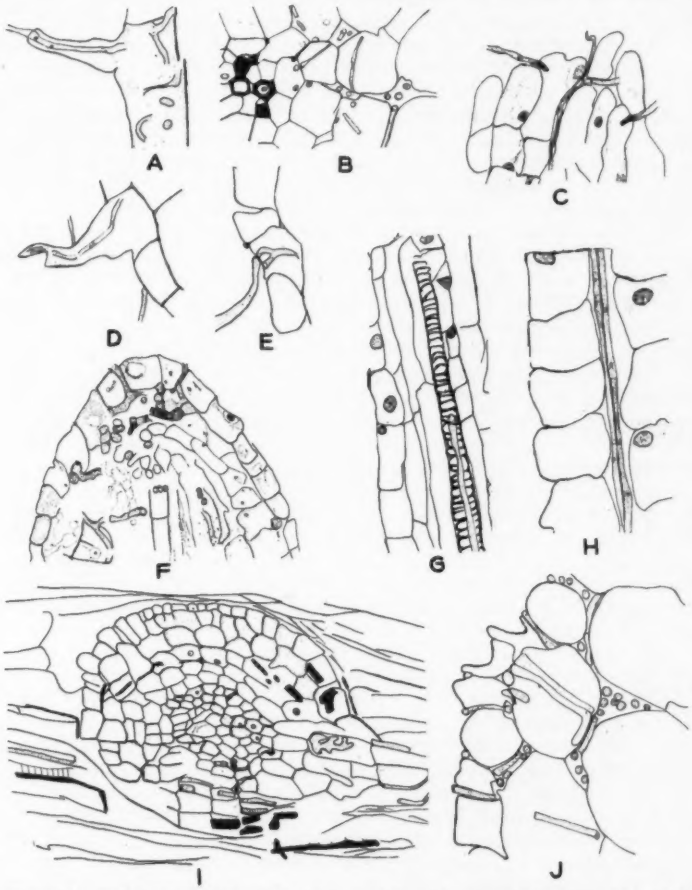


FIGURE 3.—Studies of penetration of *Fusarium conglutinans* in commercially susceptible and in homozygous susceptible cabbage grown at 24° C. on inoculated soil-extract agar. Fixations made three to eight days after inoculation: A, Fungus in an epidermal cell and its root hair. × 473. B, Fungus in cortex and stele behind the impediment of wound response. × 217. C, Hyphae in the intercellular spaces of rootcap. × 575. D, Hypha in root hair. (Preparation from C–16 × 31B.) × 473. E, Hypha entering epidermal cell. × 473. F, Hyphae entering and within primary meristem. × 473. G, Hypha within the latest differentiated tracheal vessel eight days after inoculation. × 473. H, Longitudinal section of primary meristem in the region which later differentiates into cortex. Hyphae intercellular. × 473. I, Hyphae in both primary and secondary roots. The indications are that the fungus passed from the main to the lateral root. The heavy dark areas represent suberized walls, peculiar to cortical cells in the young root of cabbage. × 217. J, Cross section of a young root. Fungus both intercellular and intracellular. (Preparation from C–16 × 31B.) × 473.

whatsoever. Tisdale (*13*) reported penetration of cabbage root hairs by *Fusarium conglutinans* in young seedlings grown in tube culture of potato agar, but he did not state how frequently it occurred.

The most important channels of fungal penetration are the embryonic region and the zone of elongation. Very soon after inoculation hyphae push their way in and out of the intercellular spaces of the rootcap and enter the primary meristem. (Fig. 3, C, F.) Infrequently the fungus enters an epidermal cell directly. (Fig. 3, E.) Behind the region of active cell division the cells of the meristem become vacuolated and enlarged, particularly in their dimension parallel with the length of the root. The fungus has a longer time to attack this part of the root and is often rather abundantly distributed in its cells and in the interspaces of the zone of elongation. Figure 3, J, shows the hyphae within the cells and in the intercellular spaces. In a longitudinal section (fig. 3, H) the hyphae are shown making their way unimpeded for a considerable distance.

Fungal growth upward in the parenchyma of the older cortical tissue might facilitate infection of lateral roots by bringing hyphae in contact with emerging tips, but such a method has not been observed. When the lateral root breaks through the primary cortex, the hyphae occasionally pass into the root rupture and invade the maturated cortex of the main root. Fungal penetration in this region, however, is of little importance. The heavily suberized walls of the inner cortical cells are an effective barrier to its inward progress, and in the natural sequence of development the parenchymatous cells of the cortex soon slough off. There is a possibility that the fungus may pass from the main root to its lateral. (Fig. 3, I.)

Fusarium conglutinans is a vascular fungus and makes it way into the protostele while that tissue is still undergoing differentiation. (Fig. 3, G.) After the innermost cortical cells become suberized the stele is barricaded and further radial ingress of the fungus is thwarted. Once within the vascular region, the fungus is more or less confined to the xylem and pushes into the aerial parts of the plant. In a few instances conidia were found in the vessels.

Cabbage is usually started in seed beds and transplanted into the field when 4 to 6 weeks old. During the transfer rootlets are frequently broken or injured. In an experiment to determine whether the fungus could penetrate injured tissue, the tips of seedlings grown under aseptic conditions were cut off. Some of these were placed in Petri dishes of soil-extract agar with inoculum directly in front of the cut portion. The checks were not inoculated. Three to five days later the exposed cells of cut ends of both inoculated and uninoculated roots were more or less collapsed and dead. Stained sections of fixed material showed the walls somewhat thickened, and both walls and lumina stained heavily with safranine. Occasionally, the fungus passed the impediment of wound response and entered the cortex and stele. (Fig. 3, B.)

In the penetration studies of cabbage seedlings grown on inoculated soil-extract agar the most important places of fungal entrance are the embryonic region of the root by way of either the rootcap or the outermost layer of cells of the primary meristem in the zones of active cell division and elongation. In addition, at times, the fungus may enter injured or broken roots, and infrequently makes its way in by the root hairs. The method of entrance is usually intercellular and infrequently intracellular.

SUBSEQUENT DEVELOPMENT

Further investigations of *Fusarium conglutinans* in relation to its host were continued on somewhat older plants grown in the soil. Seeds of HG–27A were sown in a cool greenhouse in flats of sterilized soil. Thirty days later they were transplanted to cans of artificially inoculated soil and placed in tanks kept at an approximately constant soil temperature. Cabbages grown at 18° to 22° C. exhibited slight yellowing of the lower leaves 22 to 24 days after the transfer. The yellowing became deeper and more extensive and the disease advanced. Then the younger leaves underwent a similar alteration. Four days after the first ill effects were noted some of the plants were dead. Most of them, however, remained alive from a week to 15 days. At 24° to 26° the first disease symptoms were displayed 12 and 10 days, respectively, after transplantation, and the death rate was more rapid than at the lower temperature. At 28° and 30° the plants manifested characteristic disease symptoms somewhat earlier and succumbed more quickly.

When the leaves began to yellow the plants were sectioned and fixed. The primary cortex of the root was frequently yellowed; in advanced stages of disease the vascular systems of the root and stem and occasionally the veins of the petioles and leaves were more or less browned. In general, no appreciable differences were observed in the distribution of the fungus or in its relation to the host in the plants grown at the various soil temperatures. To avoid undue repetition this report is limited to plants grown at 24° C.

The fungus was found to enter through the embryonic structures of the root and to make its way readily into the vascular system of the primary and secondary roots. Commonly, only a few hyphae were discernible, and these were usually confined to the vessels and parenchyma of the xylem. Figure 4, B, shows a longitudinal section of a young root. A hypha is seen entering at *a*, and a number of mycelial threads are distributed in the procambial region. In a cross section of a young root (fig. 4, A) the fungus is present in the vessels and parenchyma of the primary xylem. In very severe cases of infection the primary tissues as far as the procambium strand were often so disorganized that the tracheal vessels were left bare.

The distribution of the fungus in the young secondary root was similar to that already described for the primary root. In a few of the roots examined the fungus was present in both cortex and stele (pl. 1, A), but more frequently it was confined to the xylem. The hyphae were vigorous; once in a while septations were observed in them, and conidia were sometimes in evidence. (Pl. 2, A, and fig. 4, D.) In such cases the host did not exhibit any unfavorable reactions. In others the vessels were plugged with gummy material, but no definite relationship could be established between the fungus and the occlusions. (Pl. 1, D, and fig. 4, G.)

In the older taproot the invasion was generally more extensive. The fungus had had a longer time to attack the tissues and had been reinforced with mycelial threads from the lateral roots. Wood fibers, cambium, secondary xylem, and phloem had been differentiated. In many of these roots the fungus occurred in the xylem only. At times, however, in very severe cases of infection hyphae were present in all tissues of the stele. (Fig. 4, C.) Although most of the hyphae ran

parallel to the length of the root they sometimes made their way radially from one parenchymatous cell to another (fig. 4, E) or passed through the pits of reticulated vessels (fig. 4, F). Plugging of vessels in the older root did occur, but it was infrequent and could usually be traced to occluded vessels of the secondary root.

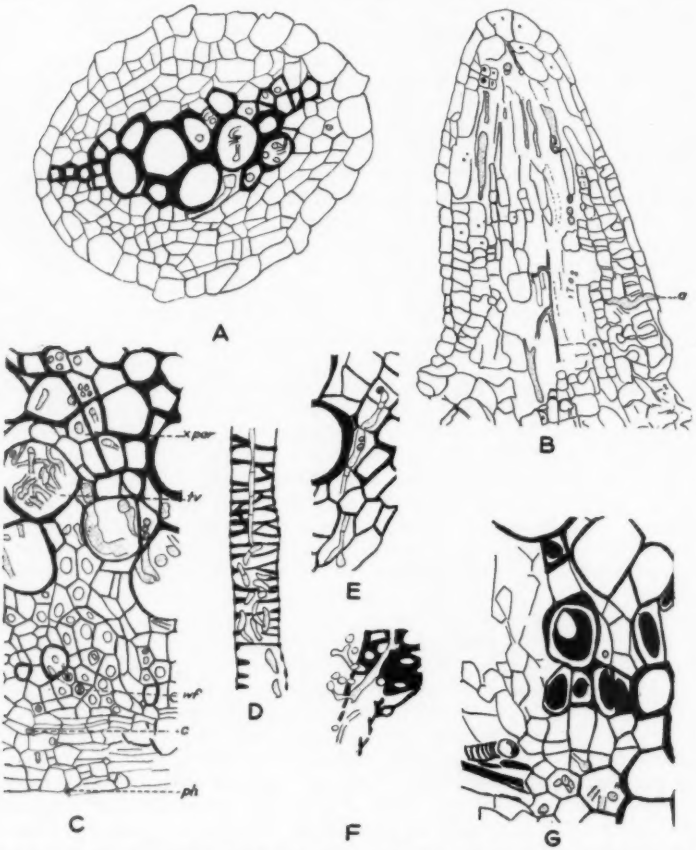
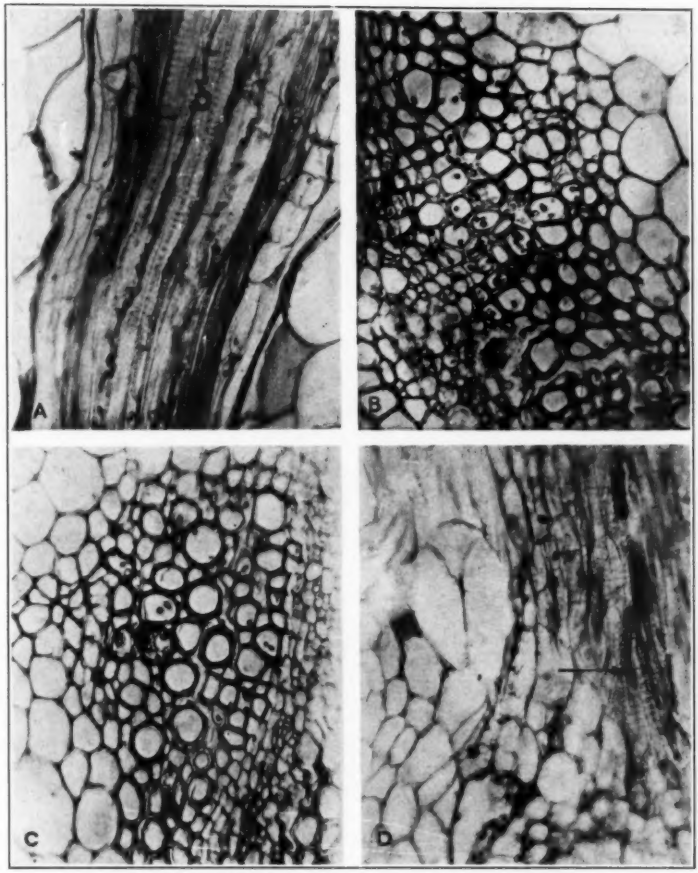


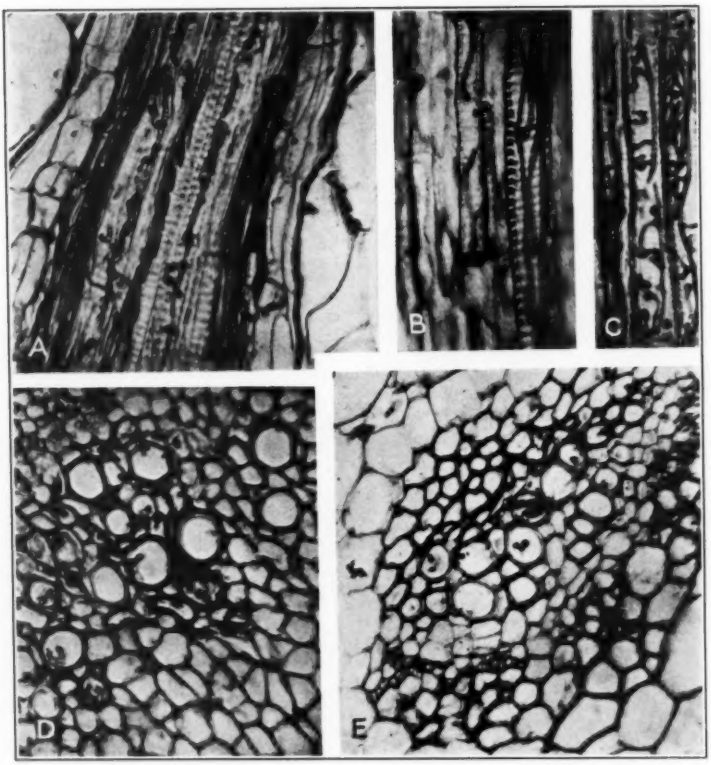
FIGURE 4.—Homozygous, susceptible cabbage transplanted to inoculated soil maintained at soil temperature 24° C. and fixed 12 to 18 days later: A, Cross section of a young root. Fungus in both vessels and parenchyma of the primary xylem. × 473. B, Longitudinal section of a young root. One hypha entered at *a*. Hyphae distributed throughout the primary meristem. × 217. C, Cross section of an older root in which secondary differentiation has taken place. Hyphae in *x par*, xylem parenchyma; *t v*, tracheal vessel; *w f*, wood fiber; *c*, cambium; and *ph*, phloem. × 473. D, Conidia in longitudinal section of a tracheal vessel. × 473. E, Hypha making its way radially in the xylem parenchyma. × 473. F, Hypha passing through pit of a reticulated vessel. × 473. G, Cross section of a taproot. Some of the vessels are plugged with a gumlike substance; some are invaded by the fungus. There is no apparent relationship between the fungus and the occlusions. × 473

In the upper hypocotyl and in the zone of transition between root and stem, the oldest part of the plant, the relative abundance of the hyphae diminished. Lateral roots were no longer bringing in additional hyphae. The fungus was confined to the tissues of the xylem. Occa-



HOMOZYGOUS SUSCEPTIBLE CABBAGE TRANSPLANTED TO INOCULATED SOIL MAINTAINED AT A SOIL TEMPERATURE OF 24° C. AND FIXED 12 TO 18 DAYS LATER. ALL X 100

A, Longitudinal section of a secondary root. Hyphae in both stele and cortex; B, cross section of young stem. Hyphae in both vessels and parenchyma of xylem; C, cross section of stem. Hyphae in both vessels and parenchyma of xylem. The walls of four of the invaded vessels are browned and the lumen of one is half filled with a gumlike substance; D, longitudinal section of a secondary root. Two of the vessels are filled with gumlike substance. There is hypha at the left of the lower occluded mass designated by the arrow.



HOMOZYGOUS SUSCEPTIBLE CABBAGE TRANSPLANTED TO INOCULATED SOIL MAINTAINED AT A SOIL TEMPERATURE OF 24° C. AND FIXED 12 TO 18 DAYS LATER. ALL X 110

A, Longitudinal section of secondary root. Conidia present in the stele; B, the hyphae extend parallel with the length of the vessel; C, both hyphae and conidia are present in the xylem elements; D, hyphae present in the xylem of the stem. Three of the invaded vessels have their walls decidedly browned; E, hyphae are established in the xylem elements of the petiole.

sionally a tracheal vessel stained yellowish red with safranine. This is the characteristic way that browned vessels react to the stain. There is practically a total absence of plugging except in the protoxylem which is being crushed out by the secondary tissues.

Most of the bundles of the stem are free of the organism. In some few, however, it occurs in the tracheae and parenchymatous cells of the xylem. (Pls. 1 and 2.) The walls of some of the vessels are frequently browned in the later stages of disease. Plugging is infrequent and seldom associated with the fungus. Occasionally the contents of one or two near-by parenchymatous cells are granular. (Pl. 1, C.) Relatively few hyphae pass into the veins of the petioles and leaves. (Pl. 2, E.) Plugging of the vessels with gumlike occlusions was occasionally observed in the petioles.

RELATION OF FUSARIUM CONGLUTINANS TO RESISTANT STRAINS OF CABBAGE

As already stated, progenies of All Head Early plants homozygous for resistance to *Fusarium conglutinans* were used. The method of studying the fungus in relation to the host is the same as that employed for the susceptible host.

PENETRATION

Seedlings grown on inoculated soil-extract agar at 24° C. were used. In the plate cultures there was no indication that a substance which was antagonistic to the fungus diffused out from the host, inasmuch as the hyphae grew in proximity to the roots, as already described for the susceptible plants. Nevertheless penetration was infrequent, as shown by the complete absence of the fungus in a large percentage of the roots sectioned. An examination of a large number of roots showed that the avenues of entrance were the same as those already described for the susceptible host. Evidence was obtained that the fungus pushed through the intercellular spaces of the rootcap (fig. 5, B), made its way between the epidermal cells (fig. 5, C), penetrated the epidermal cells directly (fig. 5, D), or entered root hairs (fig. 5, A).

The fungus also enters through wounds. The tips of a number of young seedlings were removed under aseptic conditions. Fixations were made three to five days later. The walls and cells of the injured portions stained heavily. Figure 5, J, represents the condition as observed in one inoculated root a short distance behind the incision. Some portions of the section still show the effects of the mutilation. Hyphae are present in the xylem elements.

The root of one seedling fixed four days after inoculation was an exception to the general rule. In the zone of elongation for a distance of about 1 mm. the walls of the cells in the inner portion of the cortex thickened abnormally and stained heavily with safranine. The lumina of some cells were filled with a gumlike substance, but no hyphae were in evidence. Mycelial threads were numerous about the periphery and in some instances pushed into the intercellular spaces of the epidermis and outer cortex. Not much of the invaded tissue was affected by the accumulation of gumlike substances. However, certain unusual reactions of host cells and fungus were noted and are illustrated in Figure 5, E–G. The walls of the cortical cells in a number of successive sections were thickened but no hyphae were in evi-

dence. This reaction in the root can not be explained with certainty. It may be due to the fungus. From the large number of resistant seedlings that did not respond to its presence this does not seem probable. It is more likely that the seedling was injured in transference to the inoculated agar and that the reaction is a wound response.

Generally there is little fungus in the zone of elongation. At times, however, a number of hyphae penetrate the intercellular spaces of the epidermis and enter the parenchymatous cells of the cortex. (Fig. 5, H.) The cell walls are pushed aside by the incoming fungus; but, with the exception cited, they neither thicken nor stain differently from those of the invaded tissue of the susceptible host. There is no accumulation of material in advance of the hyphae either within the cells or in the intercellular spaces. The host is apparently unaffected and the fungus appears normal.

The fungus seldom penetrates the stele of resistant seedlings grown on agar. One seedling fixed four days after inoculation showed a number of invaded vessels, one vigorous hypha extending a considerable distance in a vessel which underwent no apparent alteration. (Fig. 5, I.) The apical part of another vessel in the latest differentiated xylem of the same root was occluded. A mycelial thread was faintly distinguishable within, but it had lost its turgor and was somewhat flattened. Posteriorly in the same vessel it again appeared normal and the passage within the vessel was in no way obstructed. (Pl. 3, C.)

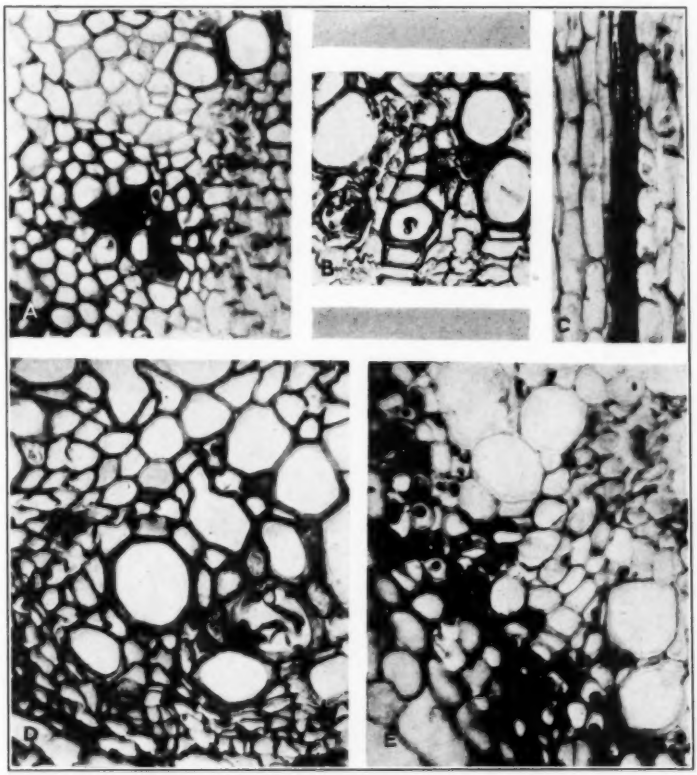
For purposes of comparison, seedlings were inoculated with a second isolation of *Fusarium conglutinans* from Racine, Wis., and with one each from Arkansas and Mississippi. A few hyphae penetrated the interspaces of the epidermis and sometimes invaded the differentiating cortex. No differences were detected in the relationship of the host and the fungus in these isolations and in that of the Racine isolation of November, 1926.

SUBSEQUENT DEVELOPMENT

Further studies of the distribution of the fungus in the resistant host were made with cabbages grown in infested soil. The method of treatment was the same as that already described for the susceptible host. When the seedlings were 1 month old they were transplanted to inoculated soil maintained at the desired temperature. Plants were fixed at various intervals 30 to 40 days later. In the earlier investigations the fungus was not detected in the many plants sectioned and stained. To facilitate the search for the presence of the fungus in the later work, thin slices from each portion being fixed were plated. Those from which the fungus was recovered were dehydrated, infiltrated with paraffin, and sectioned.

The fungus was rarely found in plants grown at soil temperatures below 28° C. and only to a limited extent in those grown at 28° and 30°. In the majority of infected plants studied the fungus was confined to the root; infrequently it extended into the stem. Wherever found, its cells were usually turgid and well supplied with protoplasm.

Most of the plants from which the fungus was recovered in only the roots displayed no external evidence of disease and exhibited no



HOMOZYGOUS RESISTANT CABBAGE. ALL X 160

A–D, Transplanted to inoculated soil maintained at a soil temperature of 28° C. and fixed 30 to 40 days later; A, two vessels and a number of parenchymatous cells in a vascular bundle of the stem plugged. The fungus does not appear to be present; B, the contents of two parenchymatous cells which contain hyphae are quite granular; C, an abnormal hypha is faintly distinguishable within the occluded vessel; D, the contents of the parenchymatous cells immediately surrounding the invaded trachea stain somewhat deeply. The unfavorable reaction may be due to the presence of the fungus; E, grown on inoculated soil-extract agar at 24° C. and fixed 8 days later. The fungus is abundant in the stele of an older root. The host shows no unfavorable response.

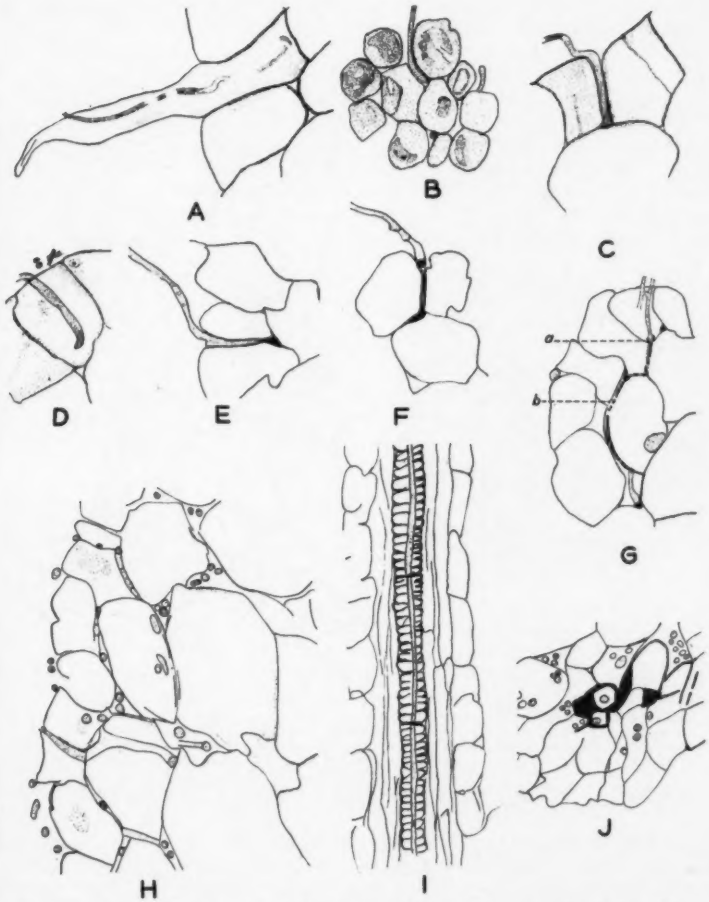


FIGURE 5.—Studies of penetration of *Fusarium conglutinans* in homozygous-resistant cabbage grown at 24° C. in inoculated soil-extract agar. Fixations made three to eight days after inoculation: A, Hypha in root hair. × 473. B, Cross section of rootcap. Hyphae in the spaces between cells being sloughed off. × 473. C, Hypha entering intercellularly. × 473. D, Hypha that has penetrated cell of outermost layer of primary meristem. × 473. E–G, Sections of a root in which there are evidences of disturbance. × 473. E, Hypha passing between two cells of the outermost layer of primary meristem and in contact with dark-staining mass. F, The hypha still outside the host. Its tip stains heavily and the walls of the cells in front of it are swollen and laminated. G, A deep-staining substance is deposited on each side of the hypha at *a*, where it meets the inner wall of the outermost layer of the primary meristem; at *b* the wall is thickened; however, the fungus is in contact with only a small part of it. H, Cross section of a young root. Fungus both intercellular and intracellular. × 473. I, Hypha within the latest differentiated tracheal vessel. × 473. J, Fungus in cortex and stele behind the impediment of wound response. × 217

symptoms of browning. Very little fungus occurred within any of them. At times it was recovered in platings, but it could not be detected in the fixed section from which the plating was made. In others one or more vessels contained a few hyphae and at times the parenchyma was invaded to a slight extent.

Various evidences of disturbance within the host were found, but none of them occurred uniformly. Invaded vessels were usually not obstructed by any gumlike substance, but at times the hyphae within seemed to form a conglomerate mass, and in a few instances gumlike deposits were associated with the pathogene. The contents of parenchymatous cells surrounding them sometimes stained more heavily or were more granular than those of neighboring cells, suggesting possibly that their protoplasm had responded to the presence of the fungus. Parenchymatous cells in which the fungus was estab-

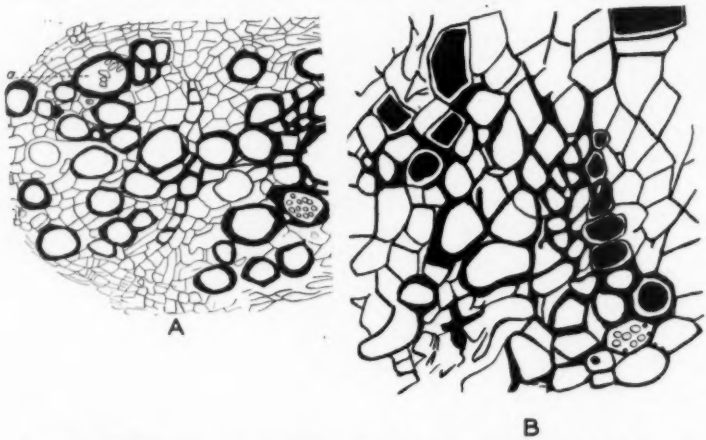


FIGURE 6.—Homozygous-resistant cabbage transplanted to inoculated soil maintained at a soil temperature of 28° C. and fixed 30 to 40 days later. Cross sections of young roots. A, Hyphae occupy one vessel of each of the xylem groups. The contents of two of the parenchymatous cells surrounding *a* are slightly granular. Note that none of the vessels are plugged with a gumlike substance. × 217. B, Cross section of a young root. Hyphae and deposits or disintegrated hyphae are present in one of the vessels. Note that the vessels of both primary and secondary xylem are plugged but that no hyphae are evident in the gumlike mass. × 473

lished usually contained but one hypha and underwent no apparent change. In some roots there was no indication of plugging; in others one or more of the xylem elements were filled with the gumlike substance. (Fig. 6.)

When the invasion was more extensive at the time of fixation, the plants often appeared somewhat stunted and the leaves showed atypical symptoms described elsewhere (*15*). Browning was present in the walls of some of the tracheal and of some xylem parenchyma cells of both root and stem. Sometimes a large part of the young taproot was disorganized and hyphae permeated all its tissues. But in the older portions of the same root where the tissues were intact they were confined to the vascular system. (Pl. 3, E.) If disturbances appear within the host they are confined largely to the growing portions of the stem. Sometimes the parenchymatous cells surrounding vessels in which the fungus is established stain more deeply than those of

neighboring cells. (Pl. 3, D.) Occasionally the contents are quite granular and massed about the hypha within. (Pl. 3, B.) At still other times the vessels in some bundles are more or less plugged in the apparent absence of the fungus. The case shown in Plate 3, A, is typical of such a condition.

DISCUSSION

The primary purposes of this investigation were to determine whether there were any apparent morphological differences between the cabbages resistant and those susceptible to *Fusarium conglutinans* and whether there were any visible reactions to the presence of the pathogene which might give a key to the basis of resistance.

Browning of vascular elements as previously reported by Jones and Gilman (*7*), Melhus, Erwin, and Van Haltern (*10*), and others is a characteristic symptom of the later stages of yellows, but it is probably in no way related to disease resistance. In the incipient stages and in cases of slight infection it is either absent or inconspicuous. Only in advanced stages of disease, such as are common to the susceptible host, does it become noticeable. At times it advances somewhat ahead of the fungus. It seems to be a response of the host to the presence of the pathogene. As is to be expected, if its formation is dependent upon the fungus it is more marked in the susceptible host.

As already noted (*15*), in the case of resistant plants grown at higher temperatures some browning of the vascular system is common in root and stem. In the majority of such cases the fungus was not recovered by plating. Rosen (*11*) has suggested in the case of cotton wilt that the fungus might produce in the soil toxins which, being absorbed by the plant, might cause browning without invasion by the parasite. In the case of resistant cabbage it is probable, judging from the histological evidence presented in this paper, that at high temperatures there may be some invasion of the root tips without further advance into the xylem. It is possible that under such circumstances the excretory products of the fungus may be carried up the xylem and result in discoloration of the latter.

The plugging of vessels and of parenchymatous cells with a gumlike substance has been reported by Jones (*6*) in the winter injury of alfalfa, and in the Fusarium wilt of cotton Fahmy (*4*) noted its occurrence in some of the smaller cells near the invaded xylem cells. Under a number of circumstances it may occur in cabbage in the absence of *Fusarium conglutinans*. Exposed cells of cut roots and more recently differentiated elements of the roots grown in sterilized soil often contain these waxlike deposits which may be the product of the coagulation of cell contents and of a protoplasmic response in adjacent living cells. Protoxylem elements in the region of transition between root and stem, and leaf traces in the normal cabbage sometimes have their passages more or less obscured because of the pressure exerted upon them.

In the cabbage seedlings grown on inoculated agar, plugging was observed in some of the invaded vessels of the resistant but not at all in the susceptible host. The comparison was limited, however, to a small number of roots in which the infection extended into the xylem. In the older cabbage the fungus is largely confined to the vessels and parenchyma of the xylem. Sometimes occlusions of gumlike material

block these structures, particularly in the younger parts of the stem and root. Observational evidence indicates that it is more frequent in the resistant than in the susceptible host grown in infested soil.

The deposit is undoubtedly a response of the host, probably of living cells. Its occurrence in the resistant seedlings grown on agar suggests the possibility that it may halt the progress of the fungus, but the fact that it occurs in the young roots and stems of both homozygous resistant and susceptible lines weakens this assumption. The evidence taken as a whole rather suggests that different disturbing agents may cause injurious effects which promote a similar response and that the accumulation of this gumlike substance is an after effect and does not necessarily represent a form of resistance.

Fusarium conglutinans seldom gains entrance into the homozygous-resistant strains of cabbage, but when it does so it enters by the same routes as in the homozygous-susceptible lines. The avenue in each is largely by way of the intercellular spaces between the outermost cells of the embryonic portions of the root. The absence of direct penetration of active cells and root hairs may be due to their turgidity or to the fact that their distended walls offer no place of lodgment for the hyphae or that their contents are unsatisfactory for the fungal requirements.

It seems hardly possible that resistance is bound up with any volatile substance emitted by the host, for hyphae develop plentifully about the seedlings grown on inoculated agar, but only occasionally penetrate. When they do enter there is, in general, no visible response of the host, which accounts for their sparsity. Evident morphological differences between the resistant and the susceptible lines which seemed to be responsible were not detected by either Tims (*12*) or the writers.

When cabbage roots are mechanically injured the living cells that are severely affected die. In general, the walls of the cells in the immediate vicinity thicken and the wounded portions heal over. Occasionally, in both resistant and susceptible strains of the host, the fungus was found established in the xylem beyond the barrier, but no differences in the response of the two types of hosts were observed. In an experiment reported in another paper (*15*, *Table 6*), the roots were severely pruned and then transplanted to infested soil. Forty-four days after transplantation the majority of the susceptible plants had succumbed to yellows, while the resistant plants showed no symptoms of disease. This brings out the fact that resistance is not confined to the embryonic portions of the root and even though the fungus may enter through wounds it does not thrive in the resistant host.

In a few instances hyphae contained yellow to brown granules, which might suggest a possible detrimental effect of host substance upon the fungus. However, they are relatively rare and are found fully as often in the susceptible as in the resistant plant.

Considerable comparative study has been made of the cell walls of plants which have strains that are susceptible and resistant to parasitic soil fungi. Tisdale (*13*) working with *Fusarium lini* of flax noted that it penetrated the cortical parenchyma of the resistant plants and that it stimulated cell division and cork formation in cells adjacent to those attacked but that the fungus was not able to invade the tissues to any extent. In a histological study of resistance of tobacco to *Thielavia basicola*, Conant (*1*) reported that under the influence of

fungal stimulation all secondary tissues of the roots of resistant plants except dead xylem elements could produce cork cells by developing a phellogen in advance of the invasion. In a cytological study of a strain of corn susceptible to *Gibberella saubinettii* grown at 12° C. on agar and inoculated at definite points on the mesocotyl, Pearson [5] found that the fungus entered through root ruptures, and although the walls of cortical cells in contact with it thickened and gumlike deposits formed in advance of it, the pathogene made its way readily through the cortex. Resistant strains grown at 12°, a temperature favorable for the development of the disease in the susceptible host, are highly resistant to the organism (*3*). By means of microchemical tests Dickson and Holbert (*2*) analyzed the nature of this resistance. They found that the cortical walls of the mesocotyl in the susceptible strain grown at 24° and of the resistant strain at 12° and 24° each formed cellulose walls impregnated with suberin, whereas those of the susceptible seedling grown under unfavorable conditions at the lower temperatures did not have walls of true cellulose but were made up of intermediate pectinlike substances which the parasite was able to dissolve and utilize as food.

The investigations reviewed were all made with tissues in which the cells were relatively more mature than that region of the cabbage root through which *Fusarium conglutinans* enters, where the tissues are largely embryonic and the cell walls are in process of formation. The parasite grows through this region and makes its way to the differentiating tracheal vessels. All the evidence indicates that resistance to the pathogene exists even in the youngest portions of the roots of the homozygous host. If this is the case, resistance in the actively growing region must be almost entirely bound up with the protoplasm. Through selection of resistant strains, physiological qualities or chemical substances antagonistic to the fungus have undoubtedly been increased or have been uniformly distributed throughout the cell.

In general, under greenhouse conditions, browning of the vascular elements is a characteristic symptom of the later stages of disease produced by *Fusarium conglutinans*. While plugging of the xylem with a gumlike substance does not occur uniformly, it seems to be a response of the host to the injurious effects of the fungus. The cytological studies made show that resistance is the result neither of morphological differences between the resistant and susceptible hosts nor of visible reactions produced by the resistant host to the presence of the parasite. Since it is present even in the meristematic region of the root where cell-wall differentiation is incomplete it may be due to the cell contents that have been altered by selection and that are adverse to fungal development within the host.

SUMMARY

Fusarium conglutinans makes its entry into the root of the cabbage intercellularly and occasionally intracellularly in the meristematic regions of the root, i. e., through the growing point and the zone of elongation, and infrequently through root hairs. In injured roots it enters through both meristematic and permanent tissue. Its progress through the root and stem is largely confined to the xylem.

[5] PEARSON, N. L. THE PARASITISM OF GIBBERELLA SAUBINETTII. [Manuscript in preparation.]

The fungus readily enters homozygous susceptible cabbage seedlings grown at 24° C. on inoculated soil-extract agar, but it penetrates only occasionally homozygous resistant strains grown under the same conditions.

Cabbage homozygous for susceptibility, 1 month or more old, grown on inoculated soil at temperatures 20°–30° C., is practically a total loss. Cabbage homozygous for resistance, of a corresponding age, grown under the same conditions, is almost completely resistant up to 24°–26°. At 28° and 30° the pathogene is largely confined to the root, but it may rarely extend into the aerial portion and bring about the death of the host.

Resistance to the fungus in strains of cabbage which have proved to be homozygous for resistance to *Fusarium conglutinans* prevails throughout the embryonic and permanent tissues of the root. A comparison of strains of cabbage homozygous for susceptibility with those homozygous for resistance does not in any way indicate that resistance is associated with morphological differences, with visible differences in wall composition, with suberization, with gumlike or other perceptible wall-occluding substances or growths, or with volatile substances. All evidence points to the conclusion that resistance in actively growing tips must be almost entirely bound up with the protoplasm and be attributed to antagonistic chemical substances or physiological qualities.

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A STUDY OF THE ACCURACY OF MEASUREMENTS OF DAIRY CATTLE[1]

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INTRODUCTION

One of the principal difficulties in genetic and nutritional experiments with domestic animals is to present in numerical terms an accurate and objective description of the animals at the beginning and during the course of the experiments. Descriptions not expressed in numerical terms are with difficulty, if at all, amenable to statistical treatment. Age and weight partially comply with the requirements for a satisfactory description, and are almost universally used. They are, however, woefully inadequate as a means of describing an animal so that a reader can reconstruct it in his own mind and see it as it actually is. For some animals, such as dairy cows, hens, fine-wool sheep, draft horses, race horses, etc., there are production records which serve as additional and very valuable descriptions. These, however, are incomplete, since animals with equal records of production often differ widely in shape, fatness, quality, and other attributes. Photographs, if taken with due regard to avoiding distortion of perspective and such photographic tricks, are accurate and objective, but it is difficult or impossible to treat them statistically even for such a simple thing as obtaining an arithmetical average.

Measurements of various parts of the animal have been used to a considerable extent as additional means of describing animals and studying changes in size and shape. Their use has been especially common in studies of dairy cattle, but they have also been used for beef cattle, horses, sheep, and even for swine. Since measurements are expressed in numerical terms, they are subject to statistical analysis. If carefully taken, they are almost purely objective, and some are exceedingly accurate. Others depend to a considerable extent upon the position of the animal, and may vary considerably with undetected variations in the pressure with which the operator applies the measuring instruments. Since the validity of the findings is dependent to a considerable degree upon the accuracy of the original measurements,[2] it was thought worth while to make a study of the extent of error normally encountered in taking measurements. Dairy cattle were used for the study. The results, noted briefly in a study[3] of beef steers, are here presented in detail.

[1] Received for publication Sept. 30, 1929; issued June, 1930.

[2] SHEWHART, W. A. CORRECTION OF DATA FOR ERRORS OF MEASUREMENT. Bell System Tech. Jour. 5: 11–26. 1926.

[3] LUSH, J. L. CHANGES IN BODY MEASUREMENTS OF STEERS DURING INTENSIVE FATTENING. Tex. Agr. Expt. Sta. Bul. 385: 17–18. 1928.

METHODS OF MEASURING

Nine Jersey cows were each measured 11 times. Twenty-five different measurements were studied. The cow stood on a level scale platform while these were being taken, and one man kept her in what was considered a normal position. Another made the measurements, and a third recorded them. All measurements were made by the same person. After one set was made, the cow was led off the platform and tied in the shade until at least two, and usually three or four other cows, had each been measured once. Then the first cow was led back on the scale platform and the second set of measurements was taken. This was repeated until 11 sets were taken for each cow. The interval between measurements gave sufficient opportunity for the cow to change her position and also for the observer to forget the figures for the previous set. The recorder was careful to make no comment on the previous measurement before the observer had announced the result of the one being taken. Since this work extended over several days, the 11 sets of records for each cow were not all taken on the same day but extended over parts of two or three days in each case, thus giving additional opportunity for temporary variations to be manifested.

EXPERIMENTAL ANIMALS

The cows were selected for measuring with two objects in mind: (1) To obtain a group which was representative so far as age was concerned of the milking herd of the station; and (2) to use where possible cows which were dry or far advanced in their lactation period, so as to interfere little with the milk production of the herd. The cows ranged in age from about 2 years and 4 months to 13 years and 3 months, most of them being near the lower limit. All were purebred or high-grade Jerseys. The measurements were made late in June, 1928.

When the data were being analyzed, the question whether the errors in measuring tended to vary with the size of the animal measured assumed considerable importance, and, in order to get more evidence on this point, 10 heifers were measured in the manner described above, 11 sets of measurements being made on each. This group of measurements was made late in December, 1928, and the time spent in measuring extended over several days. The heifers were selected so as to include all in the herd as near 1 year old as possible. Nine were between 9 and 14 months old, but 1 was only 6½ months old. All were purebred or high-grade Jerseys. The man who measured the heifers had acted as recorder when the cows were measured.

METHOD OF CALCULATION

The method of calculation follows that described by Fisher [4] under the general title Analysis of Variance, and is the same as that used in a study of the accuracy of cattle weights [5] and in a study of the accuracy of appraisals of dressed beef.[6]

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[6] LUSH, J. L., BLACK, W. H., and SEMPLE, A. T. THE USE OF DRESSED-BEEF APPRAISALS IN MEASURING THE MARKET DESIRABILITY OF BEEF CATTLE. Jour. Agr. Research 39: 147-162. 1929.

TABLE 1.—*Chest-width measurements (in centimeters) of nine cows*

Cow No.	Measurement No.—											Average
	1	2	3	4	5	6	7	8	9	10	11	
41	34.2	31.0	33.1	30.8	30.8	30.9	32.3	31.4	32.8	31.8	31.8	31.90
119	40.8	43.4	42.4	42.1	42.7	41.0	41.9	44.8	42.1	41.8	44.5	42.50
177	40.3	38.7	39.0	39.1	38.5	39.1	37.2	38.4	36.1	41.1	38.2	38.70
184	35.4	37.0	33.6	38.0	34.2	35.6	33.8	33.5	34.0	35.0	35.8	35.08
189	33.6	32.0	33.8	33.3	32.7	32.0	34.1	32.8	35.6	31.8	34.5	33.29
191	36.8	38.2	37.2	36.1	36.8	37.2	35.6	36.7	38.7	36.3	36.2	36.89
193	38.9	37.8	40.9	41.8	36.8	40.7	40.7	36.4	38.8	41.1	39.8	39.43
314	28.1	27.9	28.9	31.3	28.7	27.0	29.5	31.4	28.9	28.0	28.6	28.94
315	33.2	31.3	33.4	32.8	31.8	31.8	32.0	32.6	30.3	33.5	31.1	32.16

Table 1 shows the original data for chest width as obtained for the nine cows from the milking herd. In all, there are 99 measurements in Table 1, and the variations among these 99 items show in general two things: (1) These cows actually do differ from each other rather markedly in chest width and (2) the different measurements taken on the same cow do not agree exactly; that is, there is a certain amount of error in measuring.

TABLE 2.—*Analysis of variance in chest-width measurements of nine cows*

Cause of variation	Degrees of freedom	Standard deviation	Mean variance per degree of freedom	Total variance
		Centimeters		
All	98	4.27	18.2614	1,789.62
Differences between cows	8		204.5788	1,636.63
Inaccuracy of measurements	90	1.3	1.6999	152.99

Table 2 shows the calculations for the data presented in Table 1. The total variance (Σd^2) shown by the population of 99 items is 1,789.62. But of this total, 1,636.63 is due to the fact that the averages for the different cows were not identical, that is, to variation between cows in the group chosen for measurement. The remaining variance (152.99) is due to the fact that measurements made again on the same animal did not have exactly the same values. This latter variance may be calculated as indicated in Table 2 or by adding together the squared deviation of each of the 99 measurements from the average measurement for the cow on which it was taken. Since one degree of freedom [7] is used in fixing the mean for each cow, this total of the intra-class variance is based upon only 90 degrees of freedom. The average standard deviation of measurements taken upon the same cow is, therefore, 1.3 centimeters in the case of chest width. It includes variations due to undetected changes in the cow's position and also to errors on the part of the operator in pressing more firmly with the arms of the caliper at some times than at others. Possible errors in

[7] By "degrees of freedom" is meant the number of effective items in the population upon which the statistic in question is calculated. Since deviations are figured from the mean and one item of data is required to fix the mean, standard deviations are based in general on one less degree of freedom than there are items in the population. But when, as in this case, deviations are figured from the means of subsamples of a large population one item is used to fix each such mean and the total number of effective items will be many less than the total number of items in the population.

recording and calculating would also be included, although all such work was carefully checked to avoid errors. Corresponding to this standard error of 1.3 centimeters there can be calculated of course a "probable error" of 0.88 centimeter for the accuracy of a single measurement of chest width. These findings indicate that about half of all measurements of chest widths may deviate by as much as 0.88 centimeter more from the true measurements of the cow on which they are taken, even when as many precautions are used to insure accuracy as in these experiments. The general applicability of this finding is enhanced by the fact that the measurements were taken upon nine different cows which presumably differed in the constancy with which they assumed the same position each time they were measured. The general applicability of this finding is slightly lessened by the fact that all measurements were taken by the same operator.

Twenty-four other parts of the body were measured by the method described above, and the data thus obtained were analyzed in the same manner as the measurements of chest width, but the detailed data are omitted from this paper and only the summarized findings are presented. (Tables 3 and 4.)

TABLE 3.—*Variation shown by measurements taken on group of nine Jersey cows from milking herd*

Measurement	Average standard deviation of single measurements taken on the same cow—		Standard deviation of the mean measurements for each cow	Approximate ratio of variation between cows to error of measurement
	In actual units	As a percentage of the mean measurement of all nine cows		
	Centimeters		*Centimeters*	
Thickness of skin fold	0.032	3.91	0.10	3
Cannon circumference	.083	.54	.43	5
Length inside ear	.175	1.10	.68	4
Width at eyes	.198	.93	.55	3
Length of head	.223	.49	1.32	6
Width at pin bones	.294	1.09	1.62	6
Width at hips	.31	.67	2.78	9
Width at thurls	.36	.91	1.33	4
Width at loin	.48	1.50	1.92	4
Length of pelvis	.61	1.26	2.07	3
Depth of chest	.67	1.04	3.54	5
Muzzle circumference	.75	2.00	1.53	2
Height at knee	.79	2.38	.95	1
Greatest height at sternum	.90	1.68	2.99	3
Height over withers	.92	.77	3.01	3
Height over hips	1.09	.90	3.30	3
Height at elbow	1.09	1.57	2.01	2
Width at shoulder	1.10	2.89	4.67	4
Width of chest	1.30	3.68	4.31	3
Least height at sternum	1.30	2.52	2.15	2
Heart girth	1.37	.83	11.44	8
Paunch girth	1.78	.87	18.81	11
Flank girth	1.90	1.10	12.51	7
Length of body	2.53	1.80	4.79	2
	Square inches		*Square inches*	
Dewlap area	2.55	17.21	4.78	2

TABLE 4.—*Variation shown by measurements taken on group of 10 Jersey heifers 6½ to 14 months old*

Measurement	Average standard deviation of single measurements taken on the same heifer—		Standard deviation of the mean measurements for each heifer	Approximate ratio of variation between heifers to error of measurement
	In actual units	As a percentage of the mean measurement of all 10 heifers		
	Centimeters		*Centimeters*	
Thickness of skin fold	0.044	4.95	0.21	5
Cannon circumference	.179	1.45	.96	5
Length inside ear	.180	1.25	1.05	6
Width at eyes	.183	1.07	1.25	7
Width at hooks	.187	.63	3.76	20
Length of head	.229	.64	5.02	13
Width at pin bones	.238	1.43	2.48	10
Width at thurls	.242	.82	3.33	14
Length of pelvis	.279	.78	3.56	13
Depth of chest	.34	.72	4.35	13
Width of loin	.36	1.63	3.22	9
Muzzle circumference	.39	1.33	1.69	4
Width at shoulder	.59	2.36	3.78	6
Width at chest	.67	2.77	3.28	5
Height at knee	.68	2.19	1.49	2
Height over withers	.81	.82	6.76	8
Greatest height at sternum	.92	1.83	2.93	3
Height over hips	.93	.92	6.90	7
Least height at sternum	.98	2.01	2.44	2
Length of body	1.00	.95	10.44	10
Height at elbow	1.01	1.68	3.34	3
Chest circumference	1.13	.93	11.76	10
Flank circumference	1.74	1.41	11.96	7
Paunch circumference	2.01	1.31	14.54	7
	Square inches		*Square inches*	
Dewlap area	0.50	5.19	2.04	4

DESCRIPTION OF MEASUREMENTS STUDIED

THICKNESS OF SKIN FOLD (fig. 1, S).—This was measured with the small caliper. (Fig. 4.) The measurement was taken over the last rib, a little more than one-third of the distance down from the mid-dorsal line to the mid-ventral line. The skin was pulled out from the rib and the caliper was closed against the skin rather tightly. The measurement thus includes a double layer of skin and hair. The data were used as obtained without adjustment for this doubling.

CANNON CIRCUMFERENCE (fig. 1, V).—The steel tape (fig. 3, D) was pulled tightly around the foreleg at the smallest place between the knee and fetlock joint to obtain this measurement.

LENGTH INSIDE EAR (fig. 2, I).—One end of a steel ruler was placed against the edge of the external opening of the ear nearest the head, the distance measured being from this point to the farthest tip of the ear.

WIDTH AT EYES (figs. 1 and 2, E).—The greatest width of the head at a point about level with the eyes was measured with a small straight-arm caliper. (Fig. 3, C.)

LENGTH OF HEAD (figs. 1 and 2, A).—The extreme length of the head from the highest point on the poll to the end of the muzzle was measured with a small straight-arm caliper (fig. 3, C) in a straight line parallel to the long axis of the front surface of the head.

WIDTH AT PIN-BONES (fig. 2, N).—The calipers (fig. 3, B) were placed against the extreme lateral point of the pin bone (tuber ischii) on one side and the corresponding point on the other.

WIDTH AT HIPS (fig. 2, H).—The caliper (fig. 3, B) was placed on the extreme lateral point of the hooks (ilium) on one side and on the corresponding point on the other side.

WIDTH AT THURLS (fig. 2, J).—The points of the caliper (fig. 3, B) were placed snugly just lateral to the hip joint on each side of the pelvis.

WIDTH AT LOIN (fig. 2, L).—The points of the caliper (fig. 3, B) were placed snugly against the sides of the loin, but no more pressure was used than was necessary to make sure that the caliper points were resting against solid flesh. This measurement was taken midway between the third and fourth lumbar ver-

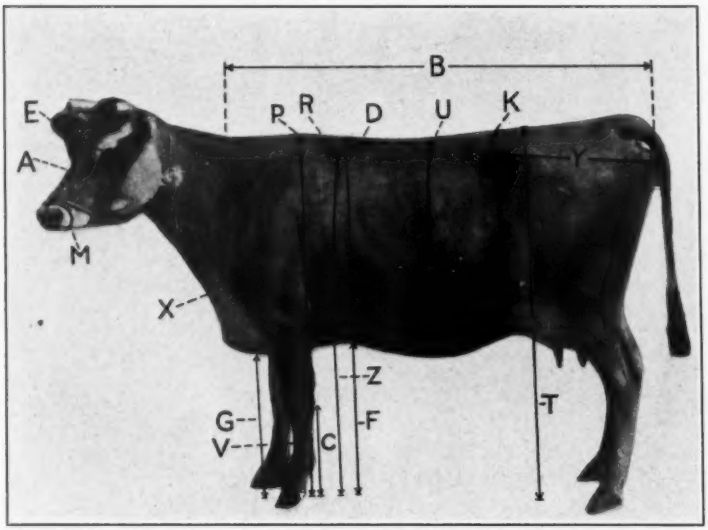

FIGURE 1.—Side view of Jersey cow with diagram showing location of various measurements studied; lettering is explained in the text

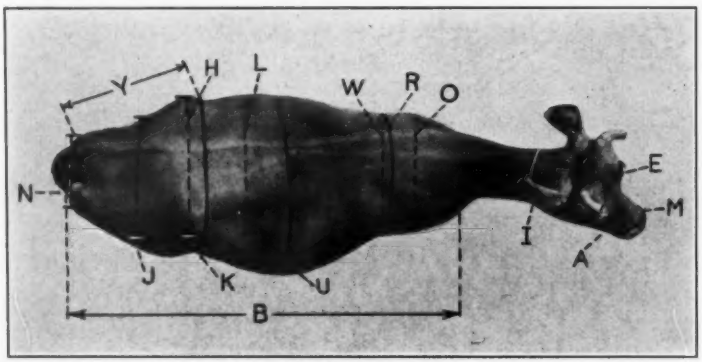

FIGURE 2.—Top view of Jersey cow with diagram showing location of various measurements studied; lettering is explained in the text

tebrae, and therefore about halfway between the forward edge of the pelvis and the rear edge of the last rib.

LENGTH OF PELVIS (figs. 1 and 2, Y).—This was the distance from the extreme posterior point of the pin bone to the extreme anterior point of the hook bone on the same side, so far as that could be located definitely on the live animal. The caliper (fig. 3, B) was used.

DEPTH OF CHEST (fig. 1, D).—This was the smallest vertical outside diameter of the chest and was measured with the parallel bars. (Fig. 3, A.)

MUZZLE CIRCUMFERENCE (figs. 1 and 2, M).—This was taken with the steel tape drawn rather tightly around the nose (including the lower jaw) at the smallest place just back of the nostrils.

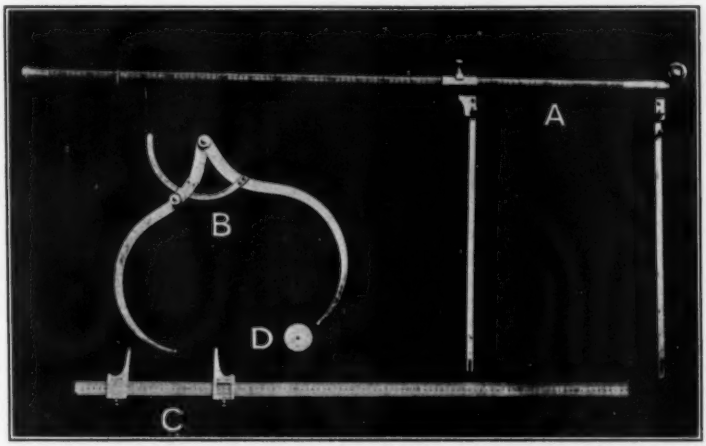

FIGURE 3.—Instruments used in measuring dairy cattle: A, Cattle-measuring standard with spirit level attached, Lydtin's model; B, caliper used for measurements of pelvic region and for width of loin; C, caliper used for measuring length of head and width at eyes; D, steel tape used for measuring girths and circumferences

FIGURE 4.—Caliper used for measuring thickness of skin

HEIGHT AT KNEE (fig. 1, C.)—This was the vertical distance from the floor to the small bony protuberance on the posterior of the knee-joint (carpus).

GREATEST HEIGHT AT STERNUM (fig. 1, F).—This was the vertical distance from the floor to the highest point on the bottom of the chest.

HEIGHT OVER WITHERS (fig. 1, P).—This was the vertical distance from the highest point over the withers to the ground.

HEIGHT OVER HIPS (fig. 1, T).—This was the vertical distance from the highest point midway between the hooks to the ground.

HEIGHT AT ELBOW (fig. 1, Z).—This was the vertical distance from the ground to the point where the superior and posterior surfaces of the ulna join.

WIDTH AT SHOULDERS (fig. 2, O).—This was the greatest transverse width of the body through the shoulders and was measured at a point 2 to 4 inches posterior to the shoulder joint.

WIDTH OF CHEST (fig. 2, W).—This was the greatest width of the chest just behind the shoulders.

LEAST HEIGHT AT STERNUM (fig. 1, G).—This was the vertical distance from the lowest part of the brisket to the ground. The caliper arm was placed snugly against the solid part of the brisket in such a way that the measurement was not influenced by the amount of dewlap present.

HEART GIRTH (figs. 1 and 2, R).—This was measured with a steel tape drawn snugly around the body in a plane perpendicular to the long axis of the body at its smallest circumference, which is just behind the front legs.

PAUNCH GIRTH (figs. 1 and 2, U).—This was the greatest circumference of the body in any plane perpendicular to the body axis.

FLANK GIRTH (figs. 1 and 2, K).—This was measured with the steel tape drawn snugly around the body at its smallest circumference just in front of the hook bones at the top of the body and as far to the rear as possible underneath the body without including any of the udder.

LENGTH OF BODY (figs. 1 and 2, B).—This was the distance in a line parallel to the main axis of the body from the extreme anterior point of the shoulder point (tuberosity of the humerus) to the extreme posterior point of the pin bone.

DEWLAP AREA (fig. 1, X).—This was the area, seen from one side, of the dewlap. The dewlap is the fold of skin along the mid-ventral line beginning just back of the muzzle and extending far back on the brisket. This area was estimated in square inches by the observer who used a ruler frequently to check his estimates. Since the dewlap area has irregular boundaries, the observer's estimation of it was not as purely objective as the other 24 measurements were.

PRESENTATION OF RESULTS

The summarized findings on the group of cows from the milking herd are presented in Table 3 and those from the group of heifers about 1 year old are shown in Table 4. In these tables the error of measuring is presented in actual units in column 2 and as a percentage of the mean of all measurements in column 3. Thus for the cows the standard error in measuring width at eyes is 0.198 centimeter, while the standard error in measuring height over withers is 0.92 centimeter, nearly five times as large. Yet the average width at eyes was 21.28, while the average height over withers was 119.4. Thus the standard error of measuring width at eyes was 0.93 per cent of the mean value of that measurement, while the standard error of measuring height over withers was only 0.77 per cent of the mean value of that measurement. The measurements are arranged in the order of their errors in actual units.

A study (now in press) pertaining in part to skin thickness on beef-bred steers showed standard deviations of 0.09 centimeter in the error of measuring skin thickness and 0.043 centimeter in the error of measuring the thicknesses of hides just after the steers had been slaughtered.

The question whether the error in measuring varies with the size of the measurement, that is, whether it tends to be a certain percentage of the measurement or a definite number of centimeters or square inches regardless of the size of the animal being measured, was studied by means of scatter diagrams made for each measurement. The average size of the measurement for each cow was plotted along one axis and the squared standard deviation of the 11 repetitions of the

measurement on the same cow was plotted along the other axis. Inspection of these diagrams showed clearly that there was for most measurements only a small correlation or no correlation at all between the average size of the measurement and the error in taking that measurement. Figures 5 and 6 show these scatter diagrams for body length and chest width, the two measurements which by inspection seemed to show the highest correlations. Figures 7 and 8 show similar diagrams for heart girth and height over withers which were picked out by inspection as representative of most of the measurements except that they showed a wider range both of averages and of squared standard deviations than most measurements did. Figure 9 shows the scatter diagram for dewlap area which differs from the other measurements in two probably important respects: (1) It is not as purely objective as the others, and (2) the heifers (which of course had smaller dewlaps than the cows even under the same conditions) were measured in cold weather (late December) while the cows were

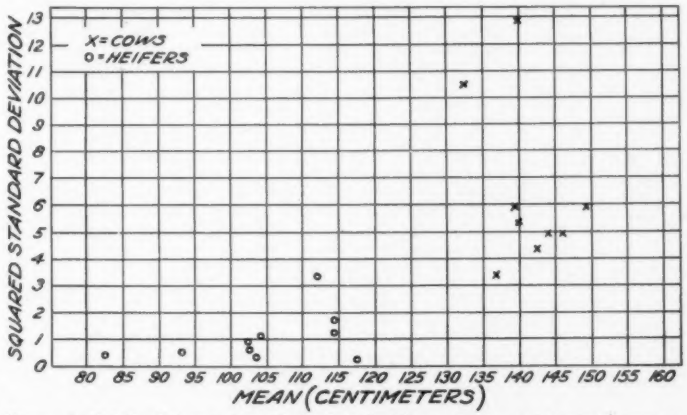

FIGURE 5.—Relation between observed standard deviation and mean size of measurements of length of body for individual animals

measured in hot weather (late June), and the dewlaps appear much smaller in cold than in warm weather. For these reasons it is no doubt better to consider separately the scatter of the data for the cows and that for the heifers, although both are shown in the same diagram.

From a study of these diagrams it may be concluded that the correlation between the size of a measurement on different animals and the usual error in taking that measurement is slight. Consequently, column 2 expresses the facts more truly than column 3 in Tables 3 and 4, although column 3 should not be entirely ignored.

Columns 4 and 5 in Tables 3 and 4 are added to show the usual importance of the error in measuring as compared to the variation between animals or "error of sampling" in selecting the groups to be measured. Column 4 is the standard deviation of the means for each cow. For example, in Table 1 the standard deviation of the figures in the last column is 4.31 centimeters, and this figure appears

in the fourth column of Table 3. Column 5 of Tables 3 and 4 is obtained by dividing the figures in column 2 into those in column 4.

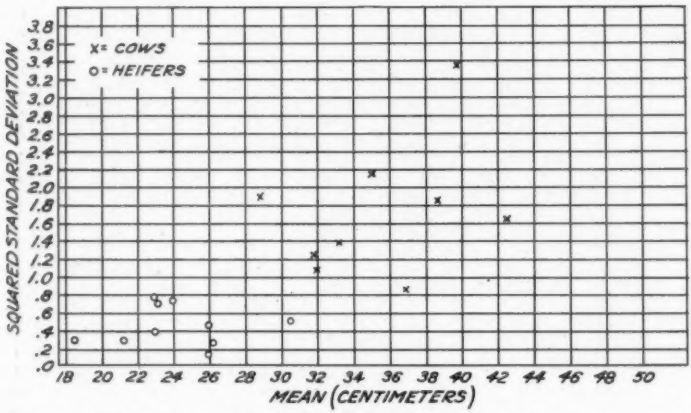

FIGURE 6.—Relation between observed standard deviation and mean size of measurements of width of chest for individual animals

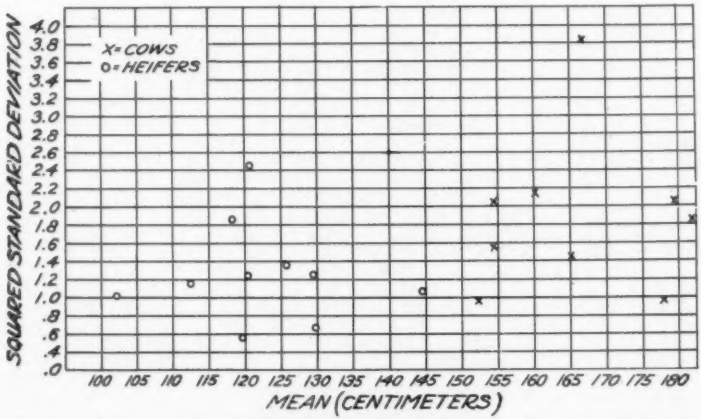

FIGURE 7.—Relation between observed standard deviation and mean size of measurements of heart girth for individual animals

Columns 4 and 5 do not have the general applicability that those in columns 2 and 3 have. The size of the figures in column 4 measures directly the heterogeneity of the group of cows on which these measurements were taken. If the group had been more uniform the figures in columns 4 and 5 would have been smaller, although the figures in columns 2 and 3 would have been changed but little if any. Conversely, less uniform groups of cows and heifers than the ones actually used would have meant larger figures in columns 3 and 4.

The figures in column 4 are not entirely free from the element of error in measuring, even though they are based upon averages of 11 measurements. However, the error of measuring is a very small part

of the figures in column 4. For example, the 0.95 centimeter standard deviation for height at knee is only reduced to 0.92 centimeter when corrected for errors of measurement.[8] For other measurements the reduction would be relatively less, since the error of measurement is a smaller part of the figure given in column 4.

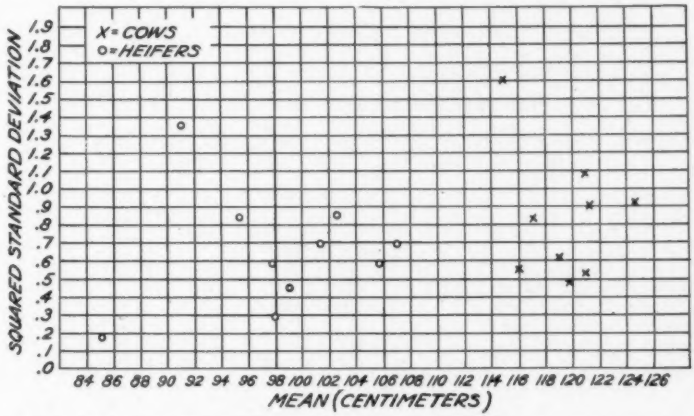

FIGURE 8.—Relation between observed standard deviation and mean size of measurements of height over withers for individual animals

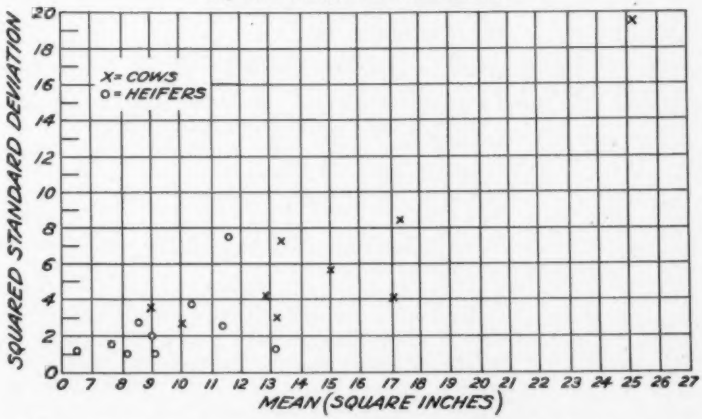

FIGURE 9.—Relation between observed standard deviation and mean size of measurements of dewlap area for individual animals

The figures in column 5 indicate that for these groups, single measurements were in most cases accurate enough to determine signifi-

[8] Shewhart's formula is as follows:

$\sigma O = \sqrt{\sigma T^2 + \sigma E^2}$ where σO = the observed standard deviation
σT = the true standard deviation free from errors of measurement
and σE = the standard deviation of the errors in measuring.

In the case of height at knee in these data this becomes

$$0.95 = \sqrt{\sigma T^2 + \frac{(.79)^2}{11}} \quad \text{whence } \sigma T = 0.92$$

cantly (in the statistical sense) which was the larger of two animals in the measurement concerned. Averages based on two or more repetitions of the measuring would of course be still more accurate. Thus Shewhart's formula becomes

$$\sigma O^2 = \sigma T^2 + \frac{\sigma E^2}{n}$$

where σO = the observed standard deviation

σT = the true standard deviation

σE = the standard deviation of the errors in measuring

and n = the number of repetitions of the measurement made on each animal.

As n is increased, σO approaches more closely to σT.

No amount of repetition of the measuring can eliminate entirely the error of measuring, but by using the figures in column 2 of Tables 3 and 4 one can make a rough correction for the influence of those errors on standard deviations and on coefficients of correlation calculated from the actual data.

Such corrections of course presuppose a random distribution of errors and are themselves subject to errors of sampling which make them only approximate on small populations. That is, there is no magic in these corrections which will rigidly eliminate the effect of errors in measurement. They do, however, provide an average correction for the bias toward large standard deviations and small correlations caused by errors of measurement.

GENERAL DISCUSSION

There is in general a close correspondence in the findings from the two groups of cattle, although the error was for most measurements distinctly larger for the larger animals. For the most part the measurements were found to be more accurate than the writers had expected. The accuracy of most of them compares rather favorably with the accuracy of weights where the standard error of weighing was found [9] in most cases to be between 6 and 12 pounds, or not far from 1 per cent of the mean weight.

Most of the measurements seemed to be less affected by day-to-day changes in weather and other external conditions than weights were, but some, notably dewlap area, and perhaps also paunch girth and flank girth, were even more affected by such changes than weight was.

The principal objection to the extensive use of body measurements, at least with dairy cattle, seems to be not their inaccuracy but their inadequacy to describe the animal in a complete way. A very large number of measurements would be necessary to permit even a rough reconstruction of the surface shape of a cow, and the mere computation of such data for a group of animals is a formidable task. Moreover, the mental process of integrating a large number of such averages into a definite mental picture of an average animal would be exceedingly difficult, if possible at all. Perhaps it is theoretically possible that there is an equation which would represent a moving point tracing out the external form of a cow in the same way that the equa-

[9] LUSH, J. L., CHRISTENSEN, F. W., WILSON, C. V., and BLACK, W. H. Op. cit.

tion $X^2+Y^2+Z^2=A$ represents the surface of a sphere with the center at the origin. But such an equation would certainly have to contain hundreds of functions of X, Y, and Z, and the mere arithmetic of its computation would be too formidable a task even for the professional mathematician. Moreover, it would change at least slightly with every movement of the cow. And when one sought to compare or average two or more such equations still greater difficulties would be encountered. Such mathematically complete descriptions of the body shape of cattle seem clearly impossible.

The taking of more and more measurements in the interest of completeness (itself not achievable) involves the law of diminishing returns of new information for each additional unit of time and money spent in collecting and analyzing the data. For most purposes a very few measurements considered in relation to each other or in relation to weight seem as much as would be really useful in contributing to the general picture of the animal and of the changes which occurred in it. Thus height over withers or over hips would certainly be included among those measurements least influenced by plane of nutrition, and therefore especially apt to be illuminating when considered in relation to weight. Depth of chest, length of body, heart girth, paunch girth, and flank girth in relation to each other and to weight all give some information about the body. So do width at shoulders, width at loin, and width of chest, although these measurements are much affected by the degree of fatness. The investigation of certain ideas current among judges of livestock may warrant the special study of measurements of head and pelvis. But with completeness of description never attainable and with the labor of computation and analysis mounting more rapidly for each additional measurement, the investigation is apt soon to reach the point where the taking of additional measurements will lead to less rather than to more findings because of the increasing likelihood that the data will not be fully analyzed or that the investigator will use all his time and energy in the analysis of the measurement data, leaving untouched other more promising lines of attack.

SUMMARY

Twenty-five different measurements were each taken 11 times on each of 9 cows and 10 yearling heifers from the Jersey herd of the Texas Agricultural Experiment Station. From these data the usual error of measuring was calculated for each measurement of the cows and of the heifers. The two groups of data agree rather closely, although most of the measurements show somewhat larger errors for the cows than for the heifers.

In only a few measurements was the standard error of measuring much larger than 2 per cent of the measurement, and in about one-third of the measurements it was less than 1 per cent. The errors of measuring are of about the same magnitude as the errors of weighing when expressed as percentages of the mean.

There was a slight correlation between the mean size of a measurement for each animal and the standard deviation of the measurements taken on that animal. This varied for different measurements, and for some measurements would have been rather large if the data for the cows and the heifers had been included in a single population.

118909—30——4

THE VITAMIN-C CONTENT OF COMMERCIALLY CANNED SAUERKRAUT, TOGETHER WITH SOME OBSERVATIONS ON ITS VITAMIN-A CONTENT [1]

By BERTHA CLOW, *Instructor*, HELEN T. PARSONS, *Associate Professor*, and INA STEVENSON, *Assistant Home Economist, Department of Home Economics, University of Wisconsin* [2]

INTRODUCTION

In a previous paper (*1*)[3] it was reported that sauerkraut obtained directly from the barrel in which fermentation took place may contain approximately one-half as much vitamin C as that found in raw cabbage. This study seemed to establish the fact that there is an appreciable amount of vitamin C in sauerkraut as it comes from the fermentation vat. The purpose of the present investigation was to determine the vitamin-C content of sauerkraut as it reaches the consumer. In the course of these studies observations were made on the vitamin-A content, and these are also included.

EXPERIMENTAL MATERIAL

Sauerkraut put up in tin was obtained from six companies of the National Kraut Packers' Association. These companies were situated in Ohio, New York, and Wisconsin. At the beginning of the experiment in the latter part of January and the first of February, 1929, each company furnished three cases (24 cans per case) of sauerkraut together with the history of the shipment. From the data received it would seem that all but two brands were from the same lot; i. e., the date of "cutting" the cabbage and the date of canning the sauerkraut were uniform for that shipment.

The range of variation in some of the more important details in the manufacture of the sauerkraut studied is given in Table 1. This table shows a wide divergence in the processes used by the manufacturers of this product at the present time.

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[1] Received for publication Dec. 7, 1929; issued June, 1930. Published with the permission of the director of the Wisconsin Agricultural Experiment Station. This study was made possible through an industrial fellowship fund given the University of Wisconsin by the National Kraut Packers' Association. The research carried out under the fund was planned and executed by members of the university staff and the work was done in the university laboratories. The participation of the National Kraut Packers' Association consisted in furnishing the funds for the payment of salaries, labor, and supplies and in contributing the samples of sauerkraut used in the tests.

[2] The authors wish to express their appreciation to Dr. A. L. Marlatt of the Department of Home Economics, to Dr. E. B. Fred of the Department of Agricultural Bacteriology, and to Dr. W. H. Peterson of the Department of Agricultural Chemistry for securing the cooperation of the National Kraut Packers' Association in this investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 64.

TABLE 1.—*Range of variation in some of the details of the processes of manufacture of the six brands of sauerkraut studied*

[These data were obtained from the various companies]

Item	Extremes of difference	
Length of time in the fermentation vat	124 days	Less than 60 days.
Extremes of temperature reported in the fermentation room [a]	70° F.	24° F.
Extremes of temperature reported in the fermentation vat [a]	77° F.	30° F.
Heating before packing:		
Time [a]	20 minutes	2 minutes.
Temperature	175° F.	85° F.
Method of packing	Machine	Hand.
Heating before sealing the cans of the machine-packed sauerkraut:		
Time	5 minutes	None.
Temperature	204° F.	Do.
Heating before sealing the cans of the hand-packed sauerkraut:		
Time	5 minutes	2 minutes.
Temperature	210° F.	114° F.
Processing after sealing the cans of the machine-packed sauerkraut	None	None.
Processing after sealing the cans of the hand-packed sauerkraut:		
Time	10 minutes	8 minutes.
Temperature	212° F.	180° F.
Acidity of sauerkraut:		
Raw	1.99	1.58.
Canned	1.5	1.

[a] This information was not given by all 6 companies.

EXPERIMENTAL METHODS

VITAMIN C

The protection method was used in these studies. The criteria for adequate protection were satisfactory growth for 60 days and absence of scurvy symptoms during this time and at autopsy. Guinea pigs were used in the experiments, and for the most part they were obtained from commercial breeders. Young guinea pigs weighing about 200 gm. were put immediately on the basal ration plus cabbage, to which they had free access. From one to five days later, depending on the size of the animals, the experimental feeding was begun. The cabbage was ground in a food chopper, a small amount (5 to 10 gm.) of sauerkraut was added to the cabbage, and the mixture was put into the basal ration. Each day the amount of sauerkraut was increased, but at no time was the quantity of cabbage in the mixture less than 5 gm. per guinea pig in the group. The animals thus gradually became accustomed to the taste of the sauerkraut, and as a rule there was no trouble in getting them to eat it when the cabbage was withdrawn entirely. Except in a few cases, where the animals were large when received from the breeder, the guinea pigs weighed about 230 to 250 gm. when they were put in individual cages. The doses of sauerkraut were mixed only in the central portion of the ration and were usually consumed immediately. The basal ration consisted of the following: Alfalfa (autoclaved 30 minutes at 15 pounds pressure), 25 per cent; rolled oats, 69 per cent; purified casein, 5 per cent. The animals that were used as positive or negative controls were given the above ration with the addition of 1 per cent sodium chloride. The ration containing the salt is the one used by Ellis, Steenbock, and Hart (*4*). The casein was purified by soaking for a week in water slightly acidified with glacial acetic acid (approximately 5 c. c. per 6 quarts of tap water). This water was changed daily.

The first experiments were made with 7½ and 5 gm. daily doses, the idea being that after these animals had been on the experimental

ration long enough to give an indication of the results, the next group of guinea pigs could be started on 10 or 2½ gm. doses. With each of the six brands of sauerkraut the 7½ gm. dose proved to be within the protection level, and thus with each brand the degree of protection on 7½, 5, and 2½ gm. daily doses was determined.

In the experiments in which 7½ and 5 gm. doses were used, fresh cans of sauerkraut were opened on Monday, Wednesday, Friday, and Saturday. The top layer was removed, ground in a food chopper, and the portions weighed out. A weighted dish which fitted into the can was put on the exposed layer of sauerkraut and pushed down so that the juice came up around it. These cans were then put into an electric refrigerator. On Tuesday and Thursday these opened cans were taken out of the refrigerator, and the top layer of sauerkraut and juice, amounting approximately to one-half the total contents of the can, was removed and discarded. The remaining sauerkraut was ground as on the previous day. The animals on the 7½ and 5 gm. levels had single doses from Monday to Friday, inclusive, and double doses on Saturday. Those on the 2½-gm. level had double doses on Monday, Wednesday, and Saturday and single doses on Friday; thus they always received sauerkraut from a freshly opened can. Although the method of keeping the opened can of sauerkraut from one day until the next would seem to eliminate the factor of oxidation, it was desired that no question should arise as to oxidation when feeding at the lower level of 2½ gm.

Before each day's dose was given the food remaining in the dish from the day before was put through a sieve that allowed the basal ration to pass through but retained the sauerkraut if any were present. It was not possible to make a quantitative estimate of the amount of sauerkraut thus remaining, but all animals which after a short period of trial failed to eat the entire dose were either discarded or shifted to a lower level.

The criteria for determining the appearance of scurvy in the guinea pigs and the degree of its severity were: Stiffness of the joints and a characteristic uneven gait in running; wincing and crying on being handled; a swelling of the wrists and arms; an awkward position of the knees held away from contact with the body; and, occasionally, the "face-ache" position. The criteria on autopsy were swelling of the arms and legs; hemorrhage of the joints, muscles, and skin; looseness or brittleness of the teeth; brittleness of the leg bones; cloudiness, hemorrhage, and beading of the ribs.

VITAMIN A

Young rats from 21 to 28 days of age weighing 40 to 45 gm. were placed on the following vitamin A low ration: Purified casein, 18 per cent; salt mixture (Osborne and Mendel), 4 per cent; agar, 2 per cent; dried brewer's yeast (irradiated), 8 per cent; cooked cornstarch, 68 per cent. The ration was similar to that of Steenbock and Coward (*9*) except for the treatment of the starch and the yeast and the substitution of another salt mixture (*7*) which was being used at the time in other rations in the laboratory, and was also used in Sherman's vitamin A low ration (*8*). The casein was extracted with hot 95 per cent alcohol for approximately 50 hours and then heated at 98° C. for a week in a drying oven. It assumed a decidedly brown tinge during the process without, however, losing its palatability. The

dried commercial brewer's yeast was irradiated under a quartz mercury vapor lamp for 10 minutes at a distance of 18 inches in order to provide a convenient and adequate source of vitamin D. The starch was cooked in boiling distilled water until translucent and was then dried and ground.

The animals were caged on wire screens in groups during the depletion period, but separately at the first symptom of eye disease. The criterion for judging the depletion of vitamin A in the rats was in all cases the appearance of pronounced sore eyes and not primarily the cessation of growth. The criteria for the response of the animal to the presence of vitamin A in the doses were both the rate of growth and the speed and completeness of the recovery from sore eyes.

The doses of sauerkraut were prepared like those for the vitamin-C determinations and were fed separately in glass caster cups placed in larger metal cups to prevent spilling. Because of the difficulty which was encountered in securing quantitative intake of the sauerkraut it was found necessary in a good many instances to collect the uneaten portion of the dose, dry it to constant weight, and calculate from determinations of the dry matter of the sauerkraut what part of the dose had actually been eaten. Doses of cabbage were prepared by removing damaged or soiled leaves from a head of cabbage and taking a slice through the head to include representative portions of both the white inner and the greenish outer leaves. Doses were weighed out from the shredded pieces of this slice.

For reasons that will later be explained only one brand of sauerkraut was used for vitamin-A determinations. Brand C was selected because a sample of this was the first to arrive in the laboratory and was ready for feeding when the animals were depleted of their vitamin A stores.

PRESENTATION OF RESULTS

The six brands of sauerkraut studied are indicated by letter and will be discussed in the order of their effectiveness as antiscorbutic substances, the most effective coming first. At least four guinea pigs and in most cases five were started on each level on each brand of sauerkraut. In one instance as many as 16 were started on a single dose. The data presented in this paper include all animals except those which were discarded for the following reasons: Failure to eat sauerkraut; refusal to eat the basal ration with resultant starvation; and the presence of complicating factors such as infected lungs or liver. Such infections could not be determined until autopsy was performed, and in several instances this was at the end of the experimental period or so near the end that there was not time enough to start other animals to replace those discarded. In the case of animals that refused to eat sauerkraut or the basal ration, it was possible to substitute others and thus keep the number in each group to at least four. In only three groups—those fed 5 gm. of brand A and 2½ gm. of brands D and E—are there less than four animals included in the final report. The total number of guinea pigs started on sauerkraut doses was 110, while the number included in this report is 80. Thus there were 30 animals started on doses which it was necessary to discard.

VITAMIN C

CONTROL EXPERIMENT (FIG. 1)

By reference to Figure 1 it will be observed that the negative control animals, which received only the basal ration, developed scurvy in 15 to 20 days. Animals Nos. 25 and 26 were fed at the beginning of the studies and Nos. 146, 147, and 148 toward the end. There was no change in the source of the supplies for the ration.

With the addition of a known source of vitamin C the basal ration is shown to be adequate for growth. Five grams of cabbage is prob-

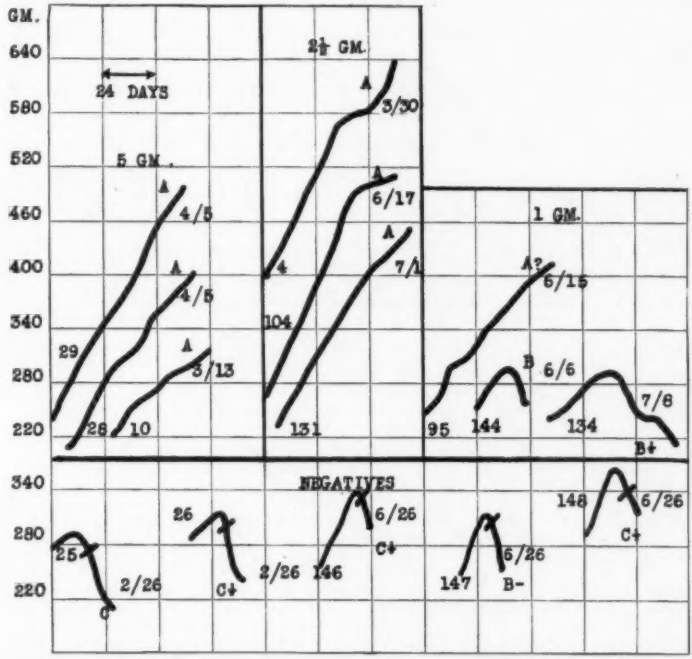

FIGURE 1.—Control experiments on vitamin C. The positive control animals received 5, 2½, and 1 gm. doses of raw cabbage daily. Animals 28 and 29 had, in addition to cabbage, varying amounts of sauerkraut chosen at random. Negative control animals were on the basal ration alone; cross lines on their weight curves indicate the point at which scurvy became evident. In all figures the numbers near autopsy indicate the month and day of the month on which autopsy was performed; autopsy symbols used as follows: A, No sign of scurvy; A?, possibility of scurvy; A−, slight but definite signs of scurvy; B+, moderate scurvy; B, B−, C+, and C, increasing degrees of scurvy

ably a very high protection level, although animal 10 did not seem to respond to the addition of this quantity with very good growth. Animals 28 and 29 were given the cabbage and sauerkraut mixture such as all the animals received during the preliminary feeding period, that is, 5 gm. of cabbage per guinea pig plus various amounts of different brands of sauerkraut chosen at random. This was done to make sure that the practice of mixing sauerkraut with the cabbage to accustom the animals to the flavor would not produce an undesirable

effect during the period before the experiment was begun. Both animals showed excellent growth and no signs of scurvy.

The 2½-gm. level of raw cabbage seems to give good growth and complete protection from scurvy. As was pointed out in a previous paper (1) this quantity of cabbage gave good growth with two animals for 44 to 58 days, but after that time there was a "flattening off" of the weight curve. In the present studies one animal, No. 104, shows a flattened curve for the last 18 days of the 60-day experimental period, while the other two animals show consistently good growth throughout the 60 days.

The 1-gm. level of feeding raw cabbage does not seem to be adequate for complete protection, although in one instance there was good

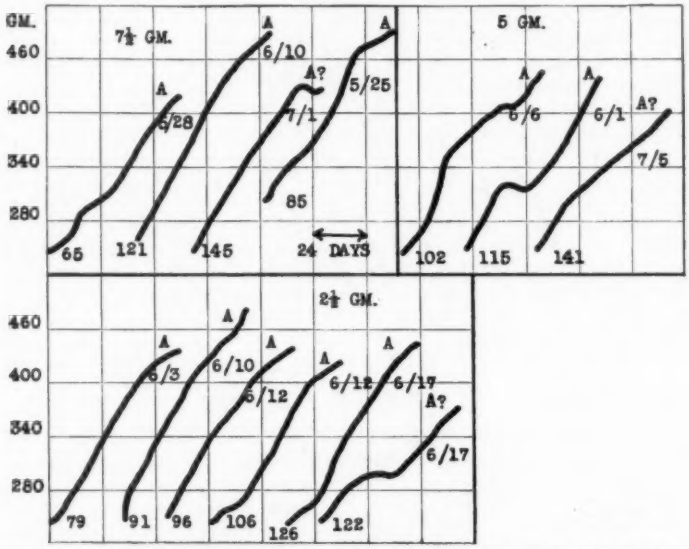

FIGURE 2.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand A. For explanation of symbols see Figure 1

growth, i. e., animal 95. (Fig. 1.) Animal 144 was chloroformed at the end of 24 days and at autopsy showed definite signs of scurvy. Animal 134, also receiving 1 gm. of cabbage, lived throughout the 60-day experimental period but showed a decline in weight after 30 days and definite symptoms of scurvy at autopsy. (Fig. 1.) It thus appears that 1 gm. of raw cabbage is the smallest quantity that will give any degree of protection.

SAUERKRAUT, BRAND A (FIG. 2)

Of all the six brands studied by far the best results were obtained with brand A. The average daily gain in weight of the four animals on the 7½-gm. level was 3.28 gm.; those on the 5-gm. level, 3.2 gm.; and those on the 2½-gm. level, 3.11 gm. Thus, as far as growth is concerned, the lowest level seems to be practically as effective as the highest. The protection from scurvy at each of the three levels is

also very good. In only three cases was there a question at autopsy as to whether complete protection had been afforded, namely, animals 145, 141, and 122 receiving 7½, 5, and 2½ gm., respectively. It will be noted that the growth of these animals was not so good as that of the others receiving the same dose of this brand of sauerkraut. It should also be noted (see autopsy dates on curves, fig. 2) that autopsies were performed on guinea pig 145 (receiving 7½ gm.) and 141 (receiving 5 gm.) three weeks or more after the other animals in the group. A new case of 24 cans of sauerkraut was opened on June 14. It is possible that the last case of material was not so good as the others, or it may have been that warm weather during the latter part of June effected a partial destruction of vitamin C in the sauerkraut both before and after the cans were opened. However, the variation in temperature in both the storage rooms and the animal laboratory

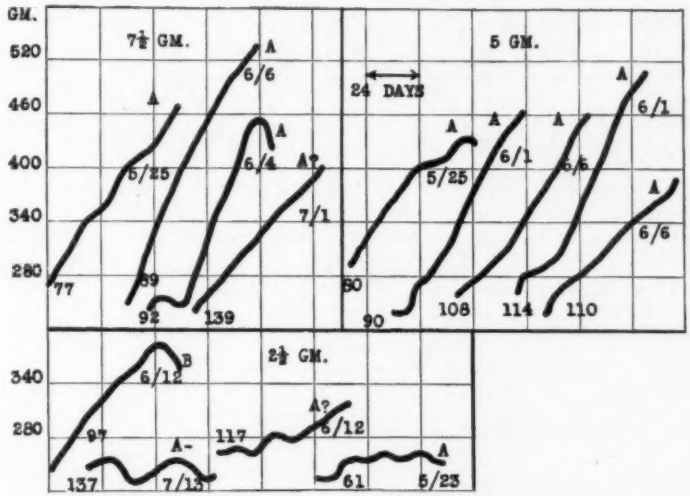

FIGURE 3.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand B. For explanation of symbols see Figure 1

was only 4° to 5° F. Further than the points made above there seems to be no obvious explanation for a condition of scurvy on the 7½ and 5 gm. doses of sauerkraut A. It seems rather significant, however, that five out of six animals receiving 2½ gm. of brand A show complete protection.

SAUERKRAUT, BRAND B (FIG. 3)

With only one exception (animal 139) the 7½ and 5 gm. levels seemed to give protection from scurvy and also very good growth, the average daily gain in weight for the group being 3.41 and 3.23 gm., respectively. Guinea pig 139, receiving 7½ gm., was probably not completely protected from scurvy. As in the case of the two animals on brand A (Nos. 145 and 141), the 60-day experimental period extended more than three weeks after that of any other animal in the group; also a new case of sauerkraut was opened on June 14. The

2½-gm. level seems to be what might be termed "the border-line dose." On such a dose the results appear to be inconsistent; for example, in this case, one animal (No. 61) maintained its weight throughout the 60 days, and on autopsy was found to be free from symptoms of scurvy; another animal (No. 97) grew very well during most of the experimental period, but autopsy showed a moderate condition of scurvy. The other two animals gave results intermediate between these. It should be noted that although there was evidence

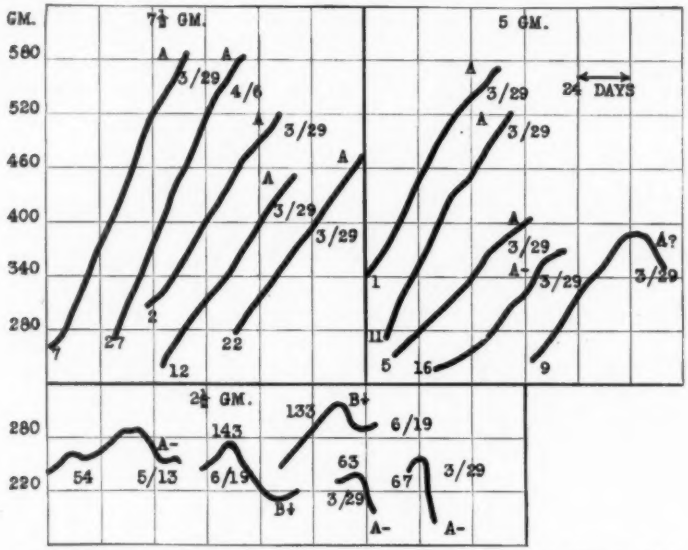

FIGURE 4.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand C. For explanation of symbols see Figure 1

of scurvy in some cases all animals lived throughout the 60-day experimental period.

SAUERKRAUT, BRAND C (FIG. 4)

The 7½-gm. level seemed to give complete protection from scurvy and also very good growth, the average daily gain in weight for the group being 4.39 gm. The 5-gm. level, although giving an average daily gain of 3.23 gm. for the group, did not give complete protection from scurvy in every case. Guinea pig 16 had a definite although slight hemorrhage on each of the hind legs. Guinea pig 9 seemed to have slightly brittle bones. Thus it can not be said that the 5-gm. level of brand C gives complete protection, but because of the fairly consistent good growth this level should doubtless be considered slightly above the "border-line" dose. The 2½-gm. level is quite definitely below the border-line dose, as in each case there was evidence of scurvy and only one animal lived 60 days.

SAUERKRAUT, BRAND D (FIG. 5)

The 7½-gm. level seems to give complete protection from scurvy and also good growth, the average daily gain in weight for the group being 3.2 gm. The 5-gm. level can probably be considered the border-line dose, as in three out of four cases there were symptoms of scurvy and two of the animals did not survive the 60-day experimental period. Although growth was good in two cases and there was complete protection in one case (No. 70), the evidence indicates that a 5-gm. level of brand D is at least very close to the border-line dose. The 2½-gm. level gave evidence of scurvy in every case, fair growth in

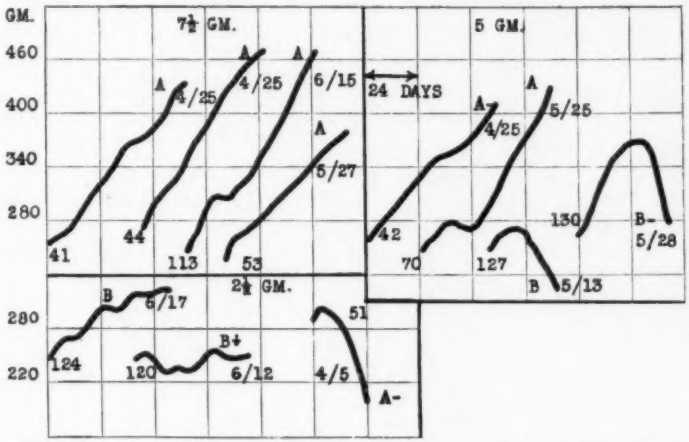

FIGURE 5.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand D. For explanation of symbols see Figure 1

one case (No. 124), and the 60-day survival period in two cases (Nos. 120 and 124).

SAUERKRAUT, BRAND E (FIG. 6)

The 7½-gm. level seems to give complete protection from scurvy and also good growth, the average daily gain in weight for the group being 3.63 gm. The 5-gm. level seemed to give good growth in four cases and fair growth in one case, the average daily gain in weight for the group being 2.96 gm. The protection from scurvy seemed to be complete in the case of two animals, while the other three on the 5-gm. level showed evidence of scurvy. The four animals on the 2½-gm. level all had scurvy, and the survival period was very short, showing that this dosage is below the protection level.

SAUERKRAUT, BRAND F (FIG. 7)

In three cases the 7½-gm. level seemed to give complete protection and also good growth, the average daily gain in weight for the animals on this dose being 3.62 gm. The fourth animal in the group (No. 135) gained weight for 36 days and then lost weight so rapidly that it was chloroformed at the end of 50 days, the autopsy showing slight but positive signs of scurvy. The 5-gm. level can probably

be considered the border-line dose, as in only two cases out of seven was there complete protection. Growth was neither consistently good nor poor. The 2½-gm. level was doubtless below the protection dose as in every case the animal had scurvy and the survival period was short, the longest being 37 days in the case of animal 58.

Another factor must be considered in evaluating sauerkraut F. The total number of animals started on doses of this brand was 28, whereas the highest total on any other brand was 19. There seemed to be difficulty in bringing the animals through a satisfactory experimental period. They did not refuse the sauerkraut, but ate very little of the basal ration after about the first 10 to 15 days, and death followed about 10 or 15 days later. Autopsy showed no scurvy, but there was usually a good deal of gas in the intestinal tract. Most

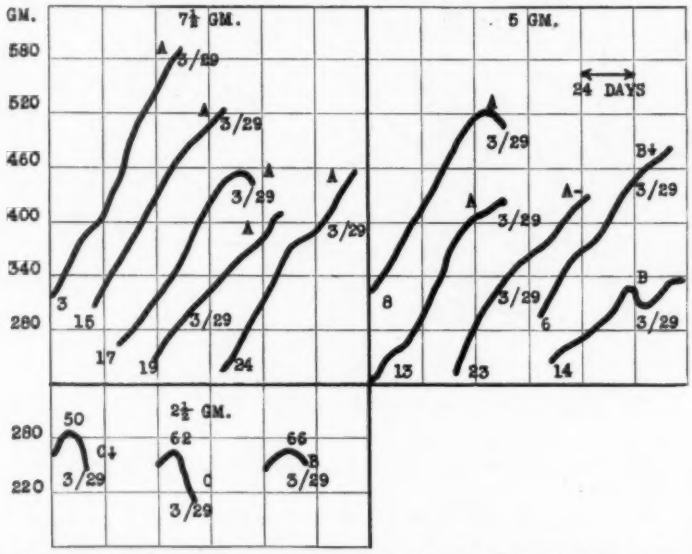

FIGURE 6.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand E. For explanation of symbols see Figure 1

of these animals were on the 5-gm. dose; in fact the failures on this level were so numerous that 16 animals were used. The writers are unable to explain these failures. Such results were not obtained with any of the other brands tested.

Comparison of Brands A to F with Fresh Raw Sauerkraut

As pointed out in the introduction, the previous work (*1*) on raw sauerkraut obtained directly from the barrel in which fermentation took place showed that 5 gm. gave complete protection, while 2½ gm. was considered the minimum protection level, i. e., the border-line dose. Two brands, namely, A and B, compare very favorably with this raw sauerkraut. Commercial brands C, D, and E are only fairly comparable, while F is probably about one-half as effective as the raw sauerkraut.

VITAMIN A

In Table 2 is presented a record of the daily doses of sauerkraut consumed by the experimental rats in addition to the basal vitamin A low ration after symptoms of depletion of vitamin A developed. As may be seen from the table, quantitative consumption of a fixed dose could be secured for only a part of the 8-week period in any of the animals. Food intake records showed that the failure of the animals to eat greater quantities of the sauerkraut could not be

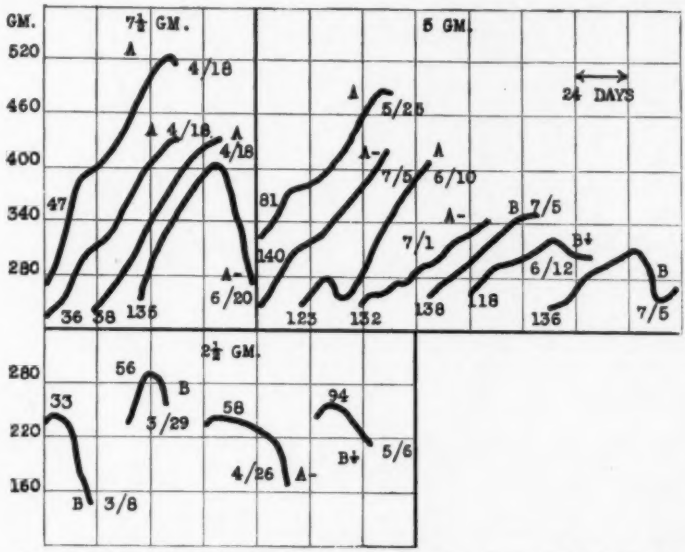

FIGURE 7.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand F. For explanation of symbols see Figure 1

attributed to lack of appetite induced by too low a consumption of vitamin B complex in the basal ration.

TABLE 2.—*Doses of sauerkraut consumed by rats following depletion of a vitamin A low diet*

[Calculations are for a preliminary period when dosage was being established; for a period when quantitative intake of fixed doses was established; for a later period when intake again fell off; and for the total period of feeding]

Rat No.	Average daily dose for preliminary period 1	Length of period	Uniform daily dose in period 2	Length of period	Average daily dose for subsequent period 3	Length of period	Average daily dose for entire period of feeding
	Grams	*Days*	*Grams*	*Days*	*Grams*	*Days*	*Grams*
16	5.4	8	7	8	4.7	41	5.0
28	4.1	18	5	20	4.2	23	4.5
29	3.5	18	5	24	2.6	19	4.1
30			(a)				3.5
32	4.2	8	6	47	4.5	6	5.6
35	2.8	10	5	15	3.4	36	3.7
36	6.5	2	12	10	5	43	6.7
39	2.8	6	4	3	2	43	2.1

[a] This rat did not at any time consume the whole of the dose weighed out.

In view of this irregularity in dosage, therefore, only a general comparison can be made between the vitamin-A content of the canned sauerkraut and that of the cabbage. The results in Figures 8 and 9 show that a daily intake of 3 to 4 gm. of stored winter cabbage leads to approximately the same gain in weight in depleted rats as that induced by an average daily intake of 4 to 6.7 gm. of

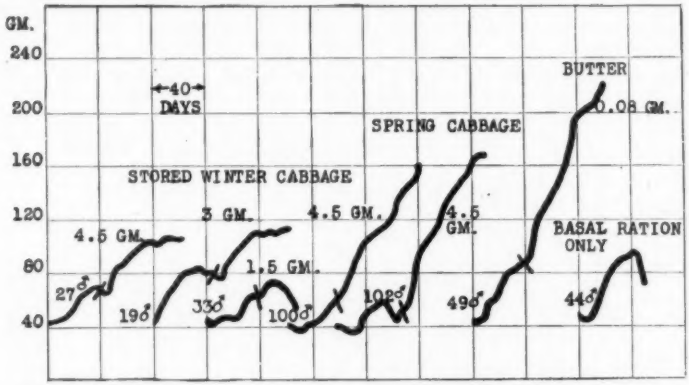

FIGURE 8.—Control experiments on vitamin A. Growth of rats during a depletion period on a vitamin-A low ration and also during a period when weighed doses of stored winter cabbage, spring cabbage, or butter were fed. Cross lines on the curves indicate the beginning of dosages. In one negative experiment the supplementary doses were not fed. For explanation of symbols see Figure 1

the canned sauerkraut. These levels are also comparable in their effect on sore eyes. This brand of sauerkraut therefore contains, roughly, one-half to three-fourths as much vitamin A as does stored winter cabbage of the type used in the manufacture of sauerkraut. Spring cabbage fed to rats 100 and 102 after the winter variety fed to the other rats was unobtainable proved to be a richer source

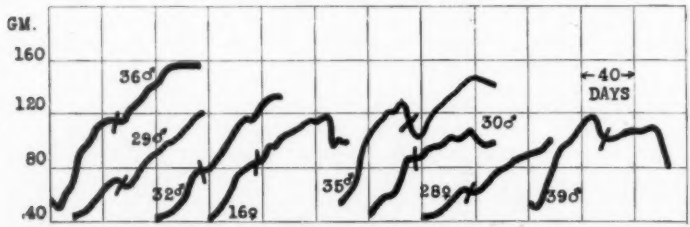

FIGURE 9.—Growth of rats during a preliminary depletion period on a vitamin-A low ration and also during a period when supplementary weighed doses of commercial sauerkraut of brand C were fed. Cross lines on the curves indicate the beginning of dosages. The intake of sauerkraut was so irregular that reference must be made to Table 2 for the size of doses consumed. For explanation of symbol see Figure 1

of vitamin A than winter cabbage, as would be expected from its smaller open head and greener color. A wide difference in the nutritive value of white and green leaves of cabbage has been noted by Hume (*5*), Delf (*3*), Coward and Drummond (*2*), and Steenbock and Sell (*10*). The practice among manufacturers of sauerkraut varies from removing only the damaged or soiled outer leaves to stripping the head until it is "white." Le Fevre (*6*, *p. 9*) states

"Sauerkraut made from green cabbage will show defects in color and texture." It is possible, therefore, that the difference in vitamin-A content noted between this brand of canned sauerkraut and the stored winter cabbage tested may be attributable largely to a difference in their content of vitamin A after stripping rather than to losses during the fermentation and canning of the sauerkraut. It should be clearly recognized, however, that the amount of vitamin A in winter cabbage is practically negligible as a source of vitamin A in the diet. For example, it would require approximately 20 to 25 pounds of the winter cabbage used in this experiment to furnish as much vitamin A as would be furnished by 1 pound of the butter used. It seems probable from the experiment that 30 to 45 pounds of the brand C canned sauerkraut studied would be needed to furnish this same amount of vitamin A.

SUMMARY

VITAMIN C

A study of the vitamin-C content of six brands of commercially canned sauerkraut has been made. The protection method was used, with guinea pigs as the experimental animals. Levels of 7½, 5, and 2½ gm. were employed. There was found to be considerable variation in the vitamin-C content of the six brands tested. Brand A was outstanding because of the good growth and protection it afforded on the 2½-gm. level. Brand B gave good growth and protection on the 7½ and 5 gm. levels but definite evidence of scurvy on the 2½-gm. level. Brand C, D, and E gave evidence of scurvy on the 5-gm. level. Brand F not only gave evidence of scurvy on the 5-gm. level but also on the 7½-gm. level. Furthermore, with brand F there was difficulty in bringing the animals through a satisfactory experimental period.

The vitamin-C content of brands A and B compare very favorably with that of specially fermented fresh raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin (*1*). C, D, and E are distinctly less effective than the raw sauerkraut, while F probably contains about one-half the amount of vitamin C found in the raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin.

Because of the lack of uniformity in the methods used in the manufacture of the sauerkraut tested, it was not possible to determine what factor or set of factors was responsible for the loss of vitamin C in the poorer brands.

No conclusions as to the probable vitamin-C content of products of the sauerkraut industry other than the sauerkraut itself put up in tin containers are warranted by these experiments.

VITAMIN A

A study of the vitamin-A content of one brand of commercially canned sauerkraut has been made with the rat as the experimental animal. The method used included the depletion of the animal's body stores of vitamin A until sore eyes appeared, and a subsequent supplying of daily weighed doses of the material to be tested in an attempt to cure sore eyes and promote normal growth. In attempting to study the comparative vitamin-A content of cabbage and

sauerkraut two difficulties in particular were encountered. Quantitative intake of doses of sauerkraut could be secured over only a part of the desired time with any of the rats, and this at levels too low to permit complete cure of the sore eyes produced during the depletion period. Difficulty was also encountered in securing satisfactory control experiments because of the unequal distribution of vitamin A in cabbage. These two limitations have made it impossible to arrive at any accurate determination of the possible losses of vitamin A during the processes of manufacture and canning of commercial sauerkraut. The results of the study of one brand suggest that differences between the vitamin-A content of sauerkraut and cabbage may be due to variations in the type of cabbage used or to the extent of the removal of the outer leaves of the head more largely than to any considerable destruction of vitamin A during the processes of fermentation and canning. The amount of vitamin A contributed to the diet by either sauerkraut or winter cabbage is practically negligible.

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POTASSIUM IODIDE AS A MINERAL SUPPLEMENT IN PAIRED FEEDING EXPERIMENTS WITH GROWING SWINE [1]

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INTRODUCTION

During recent years the advisability of reinforcing the rations of farm animals with additional minerals has received a great deal of investigation by a number of agricultural experiment stations in this country. The mineral supplements investigated, both singly and in various combinations, have been numerous. From the mass of experimental data obtained certain facts have been established beyond question. With reference to the feeding of swine, the prevalence of a calcium deficiency in the grains and their by-products has been demonstrated, and methods of correcting this deficiency, either with supplementary feeds or with mineral compounds of calcium, have been worked out. The value of salt in swine rations has been repeatedly confirmed, and the need for iodine supplements by pregnant sows in certain restricted areas has been clearly shown. Beyond these basic facts, more or less confusion prevails as regards the need for other minerals, a confusion of which commercial interests have taken the fullest advantage.

It appeared to the writers of this paper that much of this confusion about the effects of adding mineral supplements to swine rations was because of the method of experimental feeding employed. The results of the group-feeding method, particularly when no attempt is made to equalize the intake of food, are not so readily and surely interpreted as is commonly supposed, and their full explanation is in most cases impossible.

REVIEW OF LITERATURE

The situation can be well illustrated with reference to the mineral supplement with which this paper is specifically concerned, i. e., potassium iodide. An exhaustive review of the published experimental work on Iodine in Nutrition, prepared recently by Orr and Leitch (*10*),[2] removes the necessity of any extensive review of this work here. The following discussion is not concerned with the need of iodine by pregnant sows in those areas within which goiter is endemic, thus restricting the field of experimental work to which specific attention should be directed. It deals only with the possible need for iodine supplements by young growing swine in those areas of the country, including Illinois, in which goiter in farm animals is either nonexistent or of rare occurrence. Welch (*15*), as the result

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[2] Reference is made by number (italic) to "Literature cited," p. 77.

of a study of goiter in farm animals that extended over a number of years, came to the conclusion that the geographic distribution of animal goiter in this country is much more restricted than that of human goiter.

In 1925 Evvard and Culbertson (*5*) published the results of three experiments on young growing pigs concerned with the effect on growth of a potassium iodide supplement to good rations. In the first experiment the pigs were fed a basal ration of corn with a supplemental mixture containing tankage, corn gluten meal, corn oil cake meal, linseed meal, cottonseed meal, bone meal, and salt. They had access to rape pasture and additional salt. The second and third experiments involved dry-lot feeding. The basal ration in the second experiment contained corn, cottonseed meal, blood meal, limestone, salt, and bone meal; and in the third experiment it was more complicated, containing besides corn, cottonseed meal, corn oil cake meal, linseed meal, standard wheat middlings, soybean meal, peanut meal, alfalfa meal, salt, limestone, and spent bone black. In the iodide-fed lots a small amount of potassium iodide was introduced either into the supplemental feed or the supplemental minerals. The average daily intakes of potassium iodide in the three experiments were, respectively, 0.67, 0.85, and 0.08 grain per pig, or 43, 55, and 5 mgm. The experimental lots contained, respectively, 5, 7, and 6 pigs each in the three experiments.

In all experiments the rate and economy of gain averaged higher in the iodide-fed than in the control lot, and in the last two experiments the iodide-fed pigs averaged larger in length, height, and circumference. The significance of the experiment is assessed, by the authors and by those referring to it, largely on the basis of the average results only, and the inference is that the iodide supplement increased the rate of gain and decreased the cost by approximately 10 per cent. However, the probable errors of the average daily gains have been computed, and the average difference between comparable lots with their probable errors are reported for all experiments. These are, respectively, 0.103 ± 0.091, 0.126 ± 0.092, and 0.179 ± 0.052 pound. This statistical analysis affords no basis for supposing that, in the first two experiments, the greater average gain of the iodide-fed lot was the result of iodide feeding, since the average difference in gain is only slightly greater than its probable error.

The interpretation of these differences and their probable errors by the authors (*5, p. 195*) of the report may be illustrated by the statement in regard to the first experiment:

> The mean difference is therefore 1.14 times the probable error. This would lead us to expect that future experiments, similarly planned and conducted, would result in approximately 78 per cent of the cases showing that the addition of the iodide would result in an increased average gain.

Serious exception may be taken to such an interpretation. This method of analysis simply permits an estimation of the probability that the particular outcome is the result of chance only. If this probability is sufficiently small, the operation of the imposed experimental condition becomes highly probable, or even practically certain. Anything greater than such a limiting probability, which is generally taken as 1 to 30 or 1 to 50, is commonly interpreted as a negative result. The particular experiment in question is undoubt-

edly of this description. The authors (*5, p. 215*) attach considerable significance to the fact that in all three experiments the variability of the gains in the iodide-fed lot was less than that in the control lot. These facts, according to the authors, "make the beneficial results secured from iodide feeding, under Ames conditions, quite significant." The basis of this argument is not apparent.

The usual interpretation of the third experiment would be that the rates of gain of the pigs had been favorably affected by the iodide supplement. However, as Student (*1*) showed in his paper of 1908, with small samples of 10 or less the standard deviation of the population can only be roughly estimated from that of the sample, and the errors of estimation seriously affect the use of the probable error, so that greater confidence in the significance of the experimental outcome is indicated than a more critical analysis would justify. Hence, in regard to the third experiment of Evvard and Culbertson (*5*), some hesitation in accepting the usual interpretation may reasonably be urged, since there were only six pigs to the lot. It may also be pertinent to call attention to the fact that the iodide dosage in the third experiment was only one-eighth that of the first experiment and one-eleventh that of the second. As the latter doses are not at all excessive or detrimental in their physiological effect, some surprise may be felt that the dose which was by far the smallest should produce the greatest effect.

A similar analysis of the dimensional measurements is not given by Evvard and Culberston (*5*) and can not be made by the reader since the individual data are not at hand. Also, in group-feeding work, the statistical significance of average values for the economy of gains can not be assessed. It is not surprising, however, that lots showing the greater average rate of growth should also show the greater average size, as well as the greater average economy of gain. These are correlated, not independent, measurements.

In the course of a series of investigations of the mineral and vitamin requirements of pigs, Bohstedt and his associates (*2*), in their eighth experiment, tested the effect of an addition of potassium iodide to a ration of corn, flour wheat middlings, linseed meal, salt, calcium carbonate, and ferric oxide. Two groups of five young pigs each were used for this comparison. The control lot gained an average of 0.93 pound daily, and the iodide-fed lot, 1.01 pounds. The authors' statement that the iodide supplement seemed to improve the basal ration is based upon average group results, apparently without reference to individual performance.[3] Unfortunately this is the usual method of interpreting group-feeding data, though it is clearly at fault. In this experiment also, no reference is made to the average intake of food. The control pigs consumed daily an average of 4.25 pounds of the ration, while the iodide-fed pigs consumed 4.8 pounds of feed daily. Hence, the control lot did not really function as a control lot should, since it affords no information concerning the gain that would have resulted from a daily intake of 4.8 pounds of the basal ration. The lack of such information makes it impossible to

[3] The individual growth curves of the pigs in the iodide-fed lot and its control (*2, p. 214–215*) show clearly that the greater averge gain of the former lot was due to the exceptionally rapid growth of two of the five pigs in the lot.

decide how much, if any, of the average difference in rate of gain between the two lots was caused by the potassium-iodide supplement.

In 1927, Weiser and Zaitschek (*14*) of Budapest reported an experiment on swine concerned with the influence of potassium iodide administered to the dam upon the growth and health of the pigs throughout the suckling period. Forty pregnant sows were divided into two groups, the first containing 17, and the second 23 animals. The feeding and treatment of the animals in the two groups were the same except that the sows of Group 2 received daily, during the last three weeks of pregnancy and throughout the lactation period, 125 mgm. of potassium iodide. In the aggregate, the results are described as follows:

The litters in the two groups averaged initially very nearly the same in size and weight. Slightly more of the pigs in the iodide-fed group were born dead, and considerably more were crushed by the mother sow. The striking differences between the two groups to which the authors call particular attention relate to the mortality incurred and to the growth secured. During the period of observation, almost 55 per cent of the pigs in Group 1 died of disease, the nature of which was not determined. In the same period, less than 3 per cent of the pigs in Group 2 succumbed. The average growth rate of the pigs in the latter group is said to have exceeded that of the former by a considerable amount, so that at the end of the experiment the iodide-fed pigs averaged 18.54 kgm. in weight, while the control pigs averaged only 13.17 kgm.

The experiment is thus presented as a clear-cut demonstration of a markedly favorable effect of iodide feeding on growth and resistance to disease. However, there are serious objections to the manner in which the experiments were carried out. All of the litters in Group 1 were farrowed during the period from November 13 to December 23, and all of those in Group 2 were farrowed during the period from November 5 to November 14. Thus, the two groups were not under observation simultaneously, and all of the litters of Group 2 were older than the litters of Group 1 with two exceptions. The weights obtained at any definite time are not comparable, though they are treated as such with no reference to the difference in age. Thus, the average final weights already cited refer in Group 1, to litters ranging from 60 to 75 days in age, averaging 66 days, and in Group 2, to litters ranging from 74 to 83 days in age, averaging 78 days. The average weights of pigs for the surviving litters when arranged in order of their ages present a regularly descending series of values showing no break in passing from the iodide-fed to the control group, as shown in Table 1. Obviously any conclusion that the administration of potassium iodide to the sows of Group 2 affected in any way the growth of the litters is untenable. The authors of this report show an amazing disregard of the obvious explanation of the differences in weight existing among the iodide-fed and control litters, and permitted an amazing delinquency in the planning of their experiment.

TABLE 1.—*Average final weights of pigs per litter in the experiment of Weiser and Zaitschek (14), in comparison with age and iodine treatment*

Sow No.	Age of litter	Average weight per pig	Group	Sow No.	Age of litter	Average weight per pig	Group
	Days	*Kgm.*			*Days*	*Kgm.*	
1	83	12.00	Iodide.	18	76	18.88	Iodide.
2	83	19.50	Do.	19	75	15.67	Do.
3	82	20.25	Do.	1	75	15.71	Control.
4	82	21.80	Do.	2	75	9.00	Do.
5	81	18.88	Do.	20	74	14.88	Iodide.
6	81	21.00	Do.	21	74	14.33	Do.
7	80	18.29	Do.	22	74	18.30	Do.
8	80	17.88	Do.	23	74	14.88	Do.
9	79	16.33	Do.	3	70	15.67	Control.
10	78	17.67	Do.	4	70	10.86	Do.
11	78	25.17	Do.	5	63	15.00	Do.
12	78	17.13	Do.	6	63	12.00	Do.
13	78	18.82	Do.	7	61	14.86	Do.
14	77	18.78	Do.	8	60	13.83	Do.
15	77	19.00	Do.	9	60	14.00	Do.
16	76	22.67	Do.	10	60	10.00	Do.
17	76	19.14	Do.				

Consideration of the question whether the iodide administered to the sows of Group 2 protected their litters against the disease that decimated the litters in Group 1, must give due weight to the fact that over 80 per cent of the mortality in Group 1 occurred in 7 of the 17 litters, and that these 7 litters were the latest to be farrowed in that group. The 7 litters farrowed on and after December 2, containing 68 pigs at birth, were entirely destroyed before the final date of the experiment; the causes of death were not noted. Although there would be little profit in speculating on this matter, it is clear that conditions prevailing in December were very unfavorable to the growth and health of newborn pigs. Whether the supplemental iodide given the sows in Group 2 would have protected their pigs against these unfavorable conditions at a younger age can hardly be predicted from the outcome of this poorly planned experiment. The resistance of the iodide-fed pigs to disease, if one may interpret their high percentage survival [4] in this way, is but slightly different from the resistance shown by 10 of the 17 control litters.

An experiment on rats, similar in its objects to that of Weiser and Zaitschek (*14*), has been reported by Maurer and Diez (*9*), of Munich. Two groups of four pregnant rats were fed during the gestation and lactation periods upon a diet of maize and milk. In the ration of one group the iodine content of the maize had been increased by a potassium iodide fertilizer, and that of the milk by supplementing with potassium iodide the ration of the dairy cows from which it was obtained. In this way, the iodine consumed by the test animals was raised to approximately twice that of the controls; that is, 4γ [5] daily as compared with 2γ. Throughout the lactation period the body weights of the mother rats varied but little, but the average weight of the young of the mothers on the iodized ration exceeded

[4] As another instance of the partiality shown the iodide-fed group in the experiment there were 11 pigs added to the control litters at or shortly after farrowing, and 22 added to the iodide-fed litters. No mention of this is made in the text, and no allowance made in the computation of mortality or survival percentages. An admission that the pens in which the control litters were confined received less sunshine than those of the iodide-fed litters, at a time when an antirachitic factor in the food or environment is an urgent necessity, and the value of warm sanitary quarters is at a pr. mium, needs no comment.

[5] γ=0.001 milligram.

that of the young of the mothers on the normal ration, so that at the end of three weeks the average difference in weight amounted to about 20 per cent. The average weights per litter at this time were 24, 26.5, 20, and 22 gm. for the test rats, and 18.5, 21, 16.5, and 18.5 gm. for the control rats. For two other female rats, potassium iodide was added to the normal ration at the rate of 20γ daily. Neither the mothers nor their young were adversely affected by this much larger dosage of iodide, nor was the growth of the young appreciably different from that of the mothers on the untreated maize and milk. At 21 days of age the average weights of the young in these two litters were 19.5 and 21 gm. The evidence obtained in this experiment indicates a favorable effect of iodide feeding on growth at the lower level of intake, but statistically it is not competent to establish such an effect. Even less convincing are the chemical analyses of three young rats from each of four litters, two control and two test. These analyses are taken to prove profound changes in the moisture and ash contents of the rats as the result of iodide feeding.

Hanzlik, Talbot, and Gibson (*6*) have reported the results of feeding small doses of sodium iodide (about 1 mgm. daily) to young, growing rats in four experiments. The group-feeding method was employed, using three to five rats per group. The average data only are reported. The experimenters were concerned mainly with the question whether continued administration of iodide to animals will produce detrimental effects, having in mind the therapeutic treatment of thyroid troubles. Their results did not reveal any such effects, and from a consideration of the growth curves they concluded that—

> the continued administration of iodide in small daily doses in food over long periods (covering from about one-seventh to seventh-twelfths of the span of life) to rats caused moderate though variable increases in weight and growth of the body in the majority of animals on complete dietary.

Even this conservative statement may be too positive, since in the third of the four experiments the control group exceeded the iodide-fed group in rate of growth throughout the period of observation, while in the first experiment the controls ultimately exceeded the test rats in median weight.

In apparent contrast to the experiments just reviewed, which have been interpreted to mean that a supplemental feeding of iodides to animals, either directly in their food or through the milk of the dam, may exert a favorable effect upon growth and well-being, may be cited a number of other experiments in which such indications are absent. Orr and Leitch (*10*) in their review of the literature on iodine in nutrition, describe briefly a number of experiments on swine performed at the Rowett Institute at Aberdeen, Scotland, in which the effect of supplemental iodine upon growth and reproduction was investigated. The results are interpreted as entirely negative, except in so far as they showed that no toxic effects on young pigs or on brood sows are likely to follow the administration of iodine up to that equivalent to 1 gm. of potassium iodide per head per day. Essentially negative results were also secured with calves.

Rothwell (*11*) investigated the value of potassium iodide for growing swine in an experiment at the Central Experimental Farm, Canada. A lot of five pigs received daily from 50 to 100 mgm. of potassium

iodide per head. Starting at an average weight of 55 pounds, their average rate of growth to a weight of 274 pounds was somewhat less than that of the control lot.

The Purdue Agricultural Experiment Station has reported [6] the results of two years' work on the feeding of dried kelp to growing swine, with particular reference to its iodine content. Turrentine (*13*) reported the average composition of 29 samples of dried kelp from the Pacific coast, analyzed by the United States Department of Agriculture. Almost 40 per cent of this material consisted of inorganic salts, about one-half of which was sodium chloride. Its iodine content averaged 0.26 per cent and among the 29 samples ranged from 0.17 to 0.41 per cent. Turrentine voices an opinion not uncommonly held that—

> it is preferable that the body be permitted to secure its iodin from some natural, vegetable carrier of iodin, by natural digestive processes of selection and elimination through a prolonged digestive period rather than from some chemical, highly soluble and of high penetrative powers, against which the natural protective and defensive agencies of the body are not able to prevail.

That sea plants are efficient as a source of iodine is indicated by the work of Hunter and Simpson (*8*), who found the iodine content of the thyroids of sheep in the Orkney Islands, which lived largely on seaweed during the winter, was extraordinarily high (1.05 per cent on the dry basis).

The Purdue experiments were of the ordinary description and have been reported in the usual way by averages only, so that it is not possible to assess the significance of the average by any statistical method. All lots of pigs in both experiments contained 10 animals each. In the first experiment a ration of ground corn 90 and fish meal 10 promoted an average daily gain of 1.55 pounds throughout 90 days of feeding. Substitution of 1 per cent of fish meal by 1 per cent of dried kelp resulted in the same average gain, but a similar substitution of 2 per cent gave an average gain of 1.63 pounds. With tankage or soybeans as the nitrogenous concentrate, the average daily gain of the lot receiving either 1 or 2 per cent of dried kelp exceeded that of the check lot, the averages being 1.23 and 1.54 pounds for tankage, and 0.94 and 1.04 pounds for soybeans. In the second year's experiment, a ration of shelled corn and meat scraps gave an average daily gain of 1.54 pounds in a 90-day feeding trial. When dried kelp replaced one-fifth of the meat scraps, the average daily gain was 1.49 pounds, and when it was fed "free choice," the average daily gain was 1.54 pounds. If the first experiment is taken to indicate a favorable effect of iodide as a supplement to the tankage or soybean rations, the second experiment may be explained on the basis of a possible higher iodine content of the basal feeds, making an iodide supplement ineffective. It seems logical, however, to conclude that both experiments are essentially negative, the apparently positive results in the first experiment being a not greatly improbable outcome of the operation of chance only.

In an experiment reported by Cameron and associates (*3*, *4*), the feeding of sodium iodide to rats had no obvious effect upon growth, though the colloid in the thyroid gland was increased. Similarly

[6] These results have been issued only as mimeographed sheets dated Nov. 20, 1928, and Sept. 27, 1929.

Hayden, Wenner, and Rucker (7) have observed that the addition of iodine to the drinking water of young rats, subsisting upon a diet of natural foods, decreased greatly the size of the thyroid apparently without affecting the rate of growth.

In evaluating the results of the foregoing experiments, account should be taken of the fact that positive results, indicating a definite nutritive effect of a dietary factor, can not be readily disproved by negative evidence unless some error in the experiments yielding positive results can be detected, or unless it can be shown that some alternative explanation has not been disposed of. But it is equally true that an experiment can be considered to possess a definite positive significance only when it satisfies some adequate method of statistical analysis. The fact that a test group of animals shows a larger average measurement than the group carried as a control is not proof that the difference in experimental treatment between the groups is responsible, since such an outcome would result once in every two trials by the operation of chance alone. Without further statistical analysis such a result can not be considered a positive one. Unfortunately most of the above-described experiments were not planned in such a way or reported in sufficient detail so that an adequate statistical analysis can be made of them. Hence, two interpretations of them are at hand, neither of which is demonstrably more probable than the other: (1) The apparently positive results were obtained with basal rations very low in iodine or with experimental animals on the verge of iodine undernutrition, while the negative results were obtained under conditions of adequate iodine nutrition; or (2) the occurrence of apparently positive results is purely the result of chance, such as would be obtained if the iodine supplement were without effect upon growth.

METHOD

Since it appeared to the writers that the difficulty in evaluating the significance of experiments on the supplementing value of iodides for the growth of animals was inherent in the group method of feeding ad libitum, it was decided to attack the problem by an entirely different method in which the control of experimental conditions is much more complete. The paired-feeding method was therefore used. In this method, each test animal has its own control animal, selected for equality of weight, sex, breeding, and condition, and treated in every way the same, including the quantity of food consumed, except for the dietary supplement whose effect upon growth it is desired to determine. Although in equalizing the food intakes of paired animals, the intake of one or the other is restricted from time to time as its pair mate refuses some of its ration, there is no basis for the belief that the comparability of the results obtained is at all impaired, or that their significance is modified. A ration deficient in any one constituent in the support of growth, should exhibit that deficiency for all intakes of food capable of supporting growth. For the same reason, the deficiency of corn proteins in tryptophane or of a corn ration in calcium is not dependent upon the amount of food consumed, but is evident at all levels of food intake consistent with growth.

TABLE 2.—*Composition of basal ration of pigs*

Feed	Proportions in which the feeds were mixed for the pigs weighing—		
	Less than 100 pounds	From 100 to 150 pounds	Over 150 pounds
	Pounds		
Corn	76	84	92
Tankage	12	8	4
Linseed meal	6	4	2
Alfalfa meal	6	4	2
Salt	.5	.5	.5

The control animal in each pair is thus a control in all respects in so far as it is possible to make it so, with reference to its food requirements, its ability to grow, the character of the basal ration, and the amount of it consumed. Another marked advantage that the method possessed over the group-feeding method is that it capitalizes to the fullest extent the ability of the experimenter to select his animals for control and test treatment in order to assure the closest equality in performance under like conditions. The interpretation of the results by statistical methods is not in the slightest degree impaired by the most careful selection of experimental animals. However, with the group-feeding method the statistical analysis of the results is commonly made on the assumption, implied or admitted, that random selection only has been used, and in so far as the experimenter interferes with the randomness of the gains (or other results) secured, by his praiseworthy desire to balance properly his experimental groups, the applicability of the ordinary statistical method becomes less exact.

Thirteen carefully selected pairs of Poland-China pigs were used in this experiment. Their initial weights ranged from 57 to 76 pounds, though within any pair, the difference in initial weight did not exceed 4 pounds. The basal ration contained ground corn, tankage, linseed meal, alfalfa meal, and salt in proportions that were varied in accordance with the body weights of the pigs, as shown in Table 2. The chemical composition of samples of the feeds used, taken continuously throughout the experiment, is recorded in Table 3. The test on each pair was continued until at least one pig attained a weight of 175 pounds. The initial and final weights recorded in Table 4 are each the average of three weights taken on consecutive days. Intermediate weights were taken weekly on each pig. The essential data of the experiment are given in Table 4.

The potassium iodide was administered to each test pig of the 13 pairs once daily in water solution. An amount of this solution equivalent to 1 grain of elemental iodine was poured over the morning feed at feeding time.

TABLE 3.—*Chemical composition of the feeds used*

Feed	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	N-free extract	Gross energy
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Small cals. per gm.*
Corn, yellow	86.64	8.27	3.39	1.38	2.43	71.17	3,839
Tankage	93.76	55.17	11.27	23.36	.64	3.32	4,304
Linseed meal	89.95	34.44	5.05	5.39	8.91	36.16	4,229
Alfalfa meal	88.98	15.31	2.03	7.13	27.29	37.22	3,939

TABLE 4.—*Weights, gains or losses, and feed consumption of 13 pairs of pigs when one of each pair was fed potassium iodide as a mineral supplement to an otherwise adequate basal ration*

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Final weight ..... pounds..	177	184	171	177	177	180	176	164	183	175
Initial weight ..... pounds..	76	76	74	73	69	70	71	64	65	67
Total gain ..... do....	101	108	97	104	108	110	105	100	118	108
Test period ..... days..	148	148	169	169	141	141	162	162	134	134
Average daily gain ..... pounds..	.68	.73	.57	.62	.77	.78	.65	.62	.88	.81
WEEKLY GAINS OR LOSSES										
Week:										
1	−4	−3	−4	−1	−2	−3	−3	−1	1	−3
2	4	5	5	4	3	6	6	4	5	6
3	4	5	4	3	6	6	5	6	0	0
4	5	6	6	5	5	6	5	4	7	3
5	6	7	5	5	6	5	6	6	7	5
6	4	7	6	9	7	7	6	5	8	6
7	8	5	5	3	3	5	5	7	6	5
8	4	4	6	6	7	6	6	4	5	6
9	10	8	8	7	8	10	7	8	7	9
10	2	5	−2	−3	3	3	1	1	1	1
11	8	7	7	11	7	7	−4	−5	9	8
12	6	6	−10	−14	7	7	1	1	6	6
13	−4	−3	−1	3	3	5	4	1	6	3
14	2	1	0	1	5	5	0	3	5	8
15	4	6	3	7	12	9	5	2	9	9
16	3	8	4	2	7	6	6	7	9	6
17	7	2	6	6	−3	0	5	6	9	10
18	6	5	8	4	10	7	8	6	9	7
19	6	6	5	9	4	8	5	9	*9	*13
20	10	8	7	5	*10	*5	8	6	.....	.....
21	*10	*13	10	10	.....	.....	10	9	.....	.....
22	.....	.....	3	5	.....	.....	3	9	.....	.....
23	.....	.....	11	3	.....	.....	*10	*2	.....	.....
24	.....	.....	*5	*14	.....	.....	.....	.....	.....	.....
Total feed eaten ..... pounds..	493	493	543	543	519	519	504	504	494	494
Average daily ration ..... pounds..	3.33	3.33	3.22	3.22	3.68	3.68	3.11	3.11	3.68	3.68
Feed per pound of gain ..... pounds..	4.88	4.56	5.60	5.22	4.81	4.72	4.80	5.04	4.18	4.57

Item	Pair 6		Pair 7		Pair 8		Pair 9	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Final weight ..... pounds..	176	175	165	175	179	169	187	175
Initial weight ..... do....	62	63	59	61	74	74	69	67
Total gain ..... do....	114	112	106	114	105	95	118	108
Test period ..... days..	127	127	155	155	141	141	134	134
Average daily gain ..... pounds..	.90	.88	.68	.74	.74	.67	.88	.81
WEEKLY GAINS OR LOSSES								
Week:								
1	0	−1	−2	1	−3	−5	−2	−4
2	6	5	4	4	6	6	6	7
2	4	6	5	4	6	5	5	5
4	5	4	7	5	7	6	8	5
5	7	8	6	5	4	4	5	6
6	7	6	6	7	6	6	5	7
7	4	5	6	6	7	4	8	4

* 8 days in the last period.

TABLE 4.—*Weights, gains or losses, and feed consumption of 13 pairs of pigs when one of each pair was fed potassium iodide as a mineral supplement to an otherwise adequate basal ration*—Continued

WEEKLY GAINS OR LOSSES—Continued

Item	Pair 6		Pair 7		Pair 8		Pair 9	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Week—Continued.								
8	6	7	5	7	5	5	4	6
9	9	7	8	6	10	8	11	7
10	5	4	5	5	2	4	3	4
11	8	4	8	9	9	7	8	9
12	6	9	3	5	7	4	8	3
13	4	4	−6	−6	1	6	3	3
14	4	8	−6	2	7	4	4	7
15	11	6	2	1	0	2	10	8
16	7	10	6	6	2	1	9	6
17	13	11	5	8	4	6	7	9
18	*8	*9	11	4	9	6	11	6
19			7	7	5	4	*5	*10
20			7	8	*11	*12		
21			10	8				
22			*9	*12				
23								
24								
Total feed eaten—pounds	483	483	519	519	470	470	518	518
Average daily ration—do	3.80	3.80	3.35	3.35	3.33	3.33	3.86	3.86
Feed per pound of gain—do	4.24	4.31	4.89	4.55	4.48	4.95	4.39	4.79

Item	Pair 10		Pair 11		Pair 12		Pair 13	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Final weight—pounds	175	179	173	182	176	173	180	178
Initial weight—do	65	66	61	65	59	61	57	57
Total gain—do	110	113	112	117	117	112	123	121
Test period—days	141	141	169	169	162	162	162	162
Average daily gain—pound	.78	.80	.66	.69	.72	.69	.76	.75

WEEKLY GAINS OR LOSSES

Week:								
1	−4	−2	−1	−4	−4	−2	−2	−1
2	7	6	5	6	6	6	8	7
3	5	5	5	7	7	3	3	2
4	7	7	4	−1	6	6	4	4
5	4	7	3	5	6	6	3	5
6	7	6	6	4	5	7	1	1
7	5	5	4	6	6	3	4	4
8	5	4	6	7	6	6	7	6
9	8	6	10	8	8	9	8	8
10	2	6	−1	0	2	0	0	2
11	8	8	8	10	9	8	3	4
12	6	4	−3	−3	1	4	4	2
13	5	6	−1	2	0	−2	6	4
14	4	5	−2	1	0	−1	5	5
15	10	10	4	3	7	7	7	4
16	8	8	4	3	4	5	5	9
17	11	11	10	8	7	6	9	7
18	−4	−5	5	4	5	4	7	8
19	5	8	5	7	6	11	8	8
20	*11	*8	9	9	9	6	13	9
21			9	6	9	8	4	6
22			7	7	7	6	6	7
23			10	6	*5	*6	*10	*10
24			*6	*16				
Total feed eaten—pounds	520	520	561	561	571	571	529	529
Average daily ration—do	3.69	3.69	3.32	3.32	3.52	3.52	3.26	3.26
Feed per pound of gain—do	4.72	4.60	5.01	4.79	4.88	5.10	4.30	4.37

*8 days in the last period.

Reference to Table 4 shows that in 7 of the 13 pairs the control pig made the more rapid gain, while in 6 pairs the iodide-fed pig gained more rapidly. A study of the gains from week to week confirms the negative character of the experiment. Of the 277 weekly comparisons between the gains of pair mates, 143.5 favored the check pig and 133.5 favored the pig receiving the iodide supplement. The ideal result, if chance alone operated, would be 138.5 comparisons favoring either the check or the test ration. The deviation of 5 from this ideal is considerably less than the standard deviation ($\sqrt{0.5 \times 0.5 \times 277} = 8.3$), and hence is insignificant as an indication of the operation of any but chance factors, not under control.

Under the conditions of this experiment, therefore, it seems clear that potassium iodide did not increase the growth-promoting value of the basal ration.

Thus the majority of experiments concerned with the effect of iodine supplements to natural rations on the growth of young animals not obviously afflicted with hypothyroidism indicate no beneficial effect, and those experiments that have been interpreted in a positive way are either statistically inadequate or have been demonstrably misinterpreted. It may be asked whether there is any good reason to believe that, in the absence of evidences of thyroid malfunction, iodine deficiency may nevertheless exist and hence iodine administration may favorably affect growth. The following facts are pertinent to this question, though they do not afford a complete answer to it:

(1) No other function of iodine in the metabolism of the higher animals has been demonstrated than its functional relationship to the thyroid gland.

(2) Thyroid enlargement seems to be an invariable accompaniment of a dietary deficiency of iodine. The absence of a hyperplastic thyroid may thus be fairly taken as an indication of a sufficient iodine supply.

(3) In normal animals, exhibiting no evidences of thyroid enlargement and no symptoms of hypothyroidism, the administration of thyroid material by mouth has in most experimental work been associated with a retardation rather than an acceleration of growth. This is in agreement with the known stimulating effect of thyroxine upon total and nitrogenous catabolism.

(4) In hypothyroidism, the administration of iodine simulates that of thyroid material, particularly with reference to its effect on the basal metabolic rate. The effect of the administration of both thyroid material and of iodine in normal animals is less regular than in hypothyroid animals, being subject apparently to individual constitutional or functional differences. The most characteristic effect of thyroid material, that is, its stimulating effect on heat production, is generally evident with normal adult animals, though with normal children it is frequently without effect (Topper and Cohen (*12*)). Iodide administration to normal animals has been observed to increase basal metabolism, to be without effect, and to decrease it, though in all cases the changes were slight.

There is thus no compelling reason to expect that small addenda of iodide to the ration will affect animal growth in the absence of thyroid hypofunctioning, nor to assume, as Evvard and Culbertson (*5, p. 193*) do, that

> inasmuch as goitre represents fairly advanced stages of the disease it is easy to see that there may be a shortage of iodine in the ration sufficient to prevent adequate nutrition and yet not so great a deficiency as to develop goitrous pathology.

SUMMARY

A critical analysis of published experiments on the effect of supplemental iodides on the growth of animals not obviously suffering from

hypothyroidism, shows that the majority indicate no beneficial effect and that those experiments which have been interpreted in a positive way are either statistically inadequate or have been demonstrably misinterpreted.

In a paired-feeding experiment involving 13 pairs of Poland-China pigs, weighing initially from 57 to 76 pounds and finally about 175 pounds, no evidence was obtained that the administered potassium iodide, equivalent to 1 grain of iodine daily, in any way affected the rate of growth. In 7 of the 13 pairs the control pig made the more rapid gain. The basal ration used contained ground yellow corn, tankage, linseed meal, alfalfa meal, and sodium chloride.

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INFLUENCE OF SMALL QUANTITIES OF POTASSIUM IODIDE ON THE ASSIMILATION OF NITROGEN, PHOSPHORUS, AND CALCIUM IN THE GROWING PIG[1]

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PREVIOUS INVESTIGATION

In 1925, Kelly (*6*),[2] of the Rowett Research Institute at Aberdeen, Scotland, published the results of an experiment upon four growing pigs to determine the effect of potassium iodide upon the metabolism of nitrogen, calcium, and phosphorus. The results obtained were interpreted as indicating definitely that potassium iodide in the amount used increased the retention of both nitrogen and phosphorus, and less definitely of calcium. The experiment was carefully planned and executed, and the results were, for three of the four pigs, adequately controlled. In fact, for three of the pigs two methods of control were adopted, one in which each pig was a control upon itself in a preperiod and a post period of no iodide supplements, the other in which a control pig, receiving no iodide supplement at any time, was carried along on the same amount of the basal ration that the test pig received. The fourth pig was the control pig for one of the other three and was later given three increasing doses of potassium iodide in as many periods. In this case the control was hardly adequate.

With only three or four experimental animals it may be urged that the data should consistently support one interpretation before a definite conclusion is established. But in the experiment of Kelly the data are not consistent throughout. In the first experiment, the experimental pig showed greater retention of nitrogen and calcium in the potassium-iodide period than in the control period and a smaller retention of phosphorus. A markedly greater retention of all three elements occurred in the postperiod than in any of the preceding periods. However, Kelly does not use the results of this period in his interpretation because "during this period the animal had a tendency to eat its feces, thus rendering the results unreliable." The discarding of one-third of the data on this pig should be based upon something more than a casual observation of this description, particularly when there is no obvious reason why coprophagy should have been confined to one experimental animal in one experimental period.

In the second experiment, the experimental pig stored more nitrogen, calcium, and phosphorus in the potassium-iodide period than in either the preceding or the following control periods. In the third experiment, the test pig retained more of all three elements in the potassium-iodide period than in the preperiod, but less of phosphorus and of calcium than in the postperiod. The fourth experimental pig showed no distinct increase in the assimilation of nitrogen, calcium, and phosphorus in periods in which 3 or 6 mgm. of potassium iodide

[1] Received for publication Feb. 1, 1930; issued June, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 87.

were given daily, but when the dose was raised in a succeeding period to 0.5 gm. daily, an enormous increase in the storage of phosphorus, a moderate increase in the storage of nitrogen, and a slight and probably insignificant increase in the storage of calcium occurred. No postperiod was run in this case.

The interpretation that Kelly gives to his data was based less upon such relations as those just described than upon a comparison of test and control pigs, attention being almost entirely confined to the preperiods and the postassium-iodide periods. Since the data are few and are not consistent throughout, the conclusions deduced are not convincing. In explaining his conclusion that iodine administration increases nitrogen retention Kelly says, "the increased retention of nitrogen after the addition of iodine, observed in all these experiments, is an index of the inducement of better growth probably due to the stimulation of the thyroid gland by the iodine." But, as Kelly himself points out, the observed increases in nitrogen retention associated with iodide administration, were, in agreement with a number of other experiments, accompanied in general by increased urinary excretion of nitrogen and hence were generally the result of a decreased fecal excretion of nitrogen. Hence, if there is a causal relation between the observed increases in nitrogen retention and the iodine administered, one must assume that potassium iodide stimulates the digestive enzymes, or in other ways induces a more complete digestion. But a number of investigations, including those of Loevenhart and Peirce (*7*) on liver and pancreatic lipase, those of Berczeller and Freud (*1*, *2*) on malt and salivary diastase, and particularly those of Clifford (*3*, *4*, *5*) on ptyalin and pepsin, have shown with remarkable unanimity that iodides, and particularly potassium iodide, retard the action of digestive enzymes, or totally inactivate them, in all concentrations producing any effect at all. Also, Magee and Reid (*8*) have observed that the addition of small quantities of potassium iodide to the nutrient solution containing excised rabbit intestine increases the rate and amplitude of the intestinal contraction, and that the motility of the intestines from rabbits receiving iodide was greater than that of the controls. In the intact animal it is known that large doses of iodides cause diarrhea. These experimental findings are not consistent with the view that potassium iodide exerts a favorable effect upon digestion, and hence they suggest that the observed relation in Kelly's experiments between iodide dosage and decreased fecal excretion of nitrogen is not a causal one.

EXPERIMENTAL METHODS

The uncertainties that appear to exist in the interpretation of Kelly's data, and the objections that may be raised against the acceptance of his conclusions, render a repetition of the work desirable. Accordingly, the writers undertook metabolism studies on five pigs. A larger number of pigs could not be handled simultaneously with the equipment available. Hence, in order to make the most of the experiment, each pig was allowed to serve as a control upon itself in a preperiod and a postperiod in which no iodide was administered. In the test period each pig received 0.248 gm. of potassium iodide. This is the dosage used in Kelly's experiments. Each of the three periods lasted 14 days, but the collections of urine and feces were composited for analysis every 7 days. The first collection period was preceded by a

3-day period in the metabolism crates on the same amounts of food consumed during the subsequent seven days.

The pigs were crossbreds between the Poland-China and the Duroc-Jersey breeds. Their initial weights were 120, 108, 113, 111, and 108.5 pounds, respectively. Three of them were gilts and two were barrows.

The basal ration was the same in composition throughout all periods, and consisted of 79.5 parts of ground yellow corn, 15 parts of soybean-oil meal, 4 parts of linseed meal, 1 part of cod-liver oil, and 0.5 part of NaCl per 100 parts of ration. In addition, each pig received daily 5 gms. of calcium in the form of $Ca_3(PO_4)_2$. The intake of food was somewhat smaller in the fore period than in the test and after periods, but in the latter two periods it was nearly the same.

TABLE 1.—*Body weights, and food consumption of the pigs*

Period	Length of period	Initial weight	Final weight	Gain	Feed consumed daily
	Days	*Pounds*	*Pounds*	*Pounds*	*Grams*
PIG 32-30 S[a]					
Fore 1	7	120.0	131.0	11.0	1,800
Fore 2	7	131.0	138.5	7.5	1,980
Test 1	7	138.5	151.0	12.5	2,134
Test 2	7	151.0	161.5	10.5	2,160
After 1	7	161.5	175.5	14.0	2,160
After 2	7	175.5	184.5	9.0	2,160
PIG 24-3 S					
Fore 1	7	108.0	118.5	10.5	1,700
Fore 2	[b]6	118.5	122.5	4.0	1,558
Test 1	[c]5	122.5	131.5	9.0	1,836
Test 2	7	131.5	137.5	6.0	2,040
After 1	7	137.5	155.0	17.5	2,040
After 2	7	155.0	160.5	5.5	2,040
PIG 6-6 S					
Fore 1	7	113.0	127.5	14.5	1,800
Fore 2	7	127.5	135.5	8.0	1,980
Test 1	7	135.5	148.0	12.5	2,134
Test 2	7	148.0	161.5	13.5	2,160
After 1	7	161.5	172.5	11.0	2,160
After 2	7	172.5	177.5	5.0	2,160
PIG 6-9 B					
Fore 1	7	111.0	121.5	10.5	1,600
Fore 2	7	121.5	130.0	8.5	1,760
Test 1	7	130.0	138.5	8.5	1,897
Test 2	7	138.5	150.5	12.0	1,920
After 1	7	150.5	162.0	11.5	2,000
After 2	7	162.0	172.5	10.5	2,000
PIG 39-30 B					
Fore 1	7	108.5	121.0	12.5	1,800
Fore 2	7	121.0	128.5	7.5	1,980
Test 1	7	128.5	140.5	12.0	2,134
Test 2	7	140.5	152.0	11.5	2,160
After 1	7	152.0	163.5	11.5	2,160
After 2	7	163.5	175.5	12.0	2,160

[a] S=sow and B=barrow.

[b] This pig vomited part of its food on the fourth day of the period. The urine and feces were discarded for this day. For the following two days the food intake was irregular.

[c] The pig vomited on the first and fifth days of this period, and on these days the collections of urine and feces were discarded. The food intake on the second day was only 1,020 gms.

EXPERIMENTAL RESULTS

The food intakes of the pigs and the weight changes are summarized in Table 1. There is no indication that the iodide supplement during the test period affected the rate of growth of these young pigs. The average gains for the five pigs in the successive 14-day periods were 18.9, 21.6, and 21.5 pounds, respectively.

TABLE 2.—*The fecal excretion and the digestibility of nitrogen*

Period	Average daily fresh feces voided	Average daily feces on dry basis	Dry substance in fresh feces	Digestibility of nitrogen
	Grams	*Grams*	*Per cent*	*Per cent*
PIG 32-30				
Fore 1	526	189	36.0	80.2
Fore 2	622	228	36.7	76.4
Test 1	788	318	40.3	71.1
Test 2	839	286	34.1	75.3
After 1	623	265	42.4	80.0
After 2	567	215	37.8	83.5
PIG 24-3				
Fore 1	595	204	34.2	76.9
Fore 2	622	241	38.8	70.4
Test 1	498	217	43.5	75.5
Test 2	660	250	37.9	77.9
After 1	545	222	40.7	81.2
After 2	511	197	38.6	82.9
PIG 6-6				
Fore 1	628	212	33.7	80.5
Fore 2	771	258	33.4	78.4
Test 1	787	231	29.4	82.8
Test 2	701	258	36.8	82.0
After 1	580	165	28.4	88.8
After 2				
PIG 6-9				
Fore 1	551	196	35.5	78.4
Fore 2	728	219	30.0	77.4
Test 1	705	251	35.6	78.9
Test 2	615	219	35.6	82.6
After 1	542	221	40.8	82.0
After 2	503	164	32.8	85.6
PIG 39-30				
Fore 1	590	211	35.8	77.7
Fore 2	770	259	33.6	74.3
Test 1	772	278	36.0	73.4
Test 2	668	243	36.4	78.7
After 1	535	216	40.4	79.0
After 2	575	197	34.3	82.1

In Table 2 the data concerning the fecal excretion and the digestibility of nitrogen are given. In successive 7-day periods the average daily weights of dry feces were 202, 241, 259, 251, 218, and 193 gm.; the average percentages of dry matter in the feces were 35.0, 34.5, 37.0, 36.2, 38.5, and 35.8; and the average coefficients of digestibility of nitrogen were 78.7, 75.4, 76.3, 79.3, 82.2, and 83.5. In each series of values the last figure is the average of four instead of five pigs,

since the last collection of feces for pig 6–6 was not analyzed, the corresponding urine collection having been thrown away by mistake. The data do not indicate any clearly evident effects of the iodide feeding, though they suggest that the weight of dry feces may have been somewhat increased and the percentage of moisture somewhat decreased in the test periods.

TABLE 3.—*Nitrogen balances of the pigs*

[All weights in grams]

PIG 32-30

Period	Intake	Excreted in—		Total output	Balance
		Feces	Urine		
Fore	38.97	7.71	17.39	25.10	+13.87
	42.87	10.10	22.01	32.11	+10.76
Test	46.21	13.36	23.00	36.36	+9.85
	46.76	11.53	23.18	34.71	+12.05
After	46.76	9.33	22.38	31.71	+15.05
	46.64	7.72	21.65	29.37	+17.27

PIG 24-3

Fore	36.80	8.49	18.04	26.53	+10.27
	33.74	9.99	15.71	25.70	+8.04
Test	39.75	9.75	20.13	29.88	+9.87
	44.17	9.78	23.30	33.08	+11.09
After	44.17	8.29	22.75	31.04	+13.13
	44.05	7.54	24.99	32.53	+11.52

PIG 6-6

Fore	38.97	7.60	11.34	18.94	+20.03
	42.97	9.25	22.68	31.93	+11.04
Test	46.21	7.96	21.26	29.22	+16.99
	46.76	8.41	21.43	29.84	+16.92
After	46.76	5.26	13.08	18.34	+28.42

PIG 6-9

Fore	34.64	7.49	16.80	24.29	+10.35
	38.10	8.62	17.51	26.13	+11.97
Test	41.08	8.65	20.28	28.93	+12.15
	41.57	7.23	21.15	28.38	+13.19
After	43.30	7.80	20.31	28.11	+15.19
	41.76	6.02	19.53	25.55	+16.21

PIG 39-30

Fore	38.97	8.68	19.30	27.98	+10.99
	42.87	11.01	19.90	30.91	+11.96
Test	46.20	12.31	23.38	35.69	+10.51
	46.76	10.00	22.08	32.08	+14.68
After	46.76	9.81	23.41	33.22	+13.54
	46.64	8.36	19.88	28.24	+18.40

TABLE 4.—*Calcium balances of the pigs*

[All weights in grams]

Period	Intake	Excreted in—		Total output	Balance
		Feces	Urine		
PIG 32-30					
Fore	5.63	2.28	0.06	2.34	+3.29
	5.76	2.76	.07	2.83	+2.93
Test	5.87	3.80	.07	3.87	+2.00
	5.89	3.97	.03	4.00	+1.89
After	5.89	4.10	.04	4.14	+1.75
	5.87	3.44	.06	3.50	+2.37
PIG 24-3					
Fore	5.56	2.69	0.13	2.82	+2.74
	5.45	3.18	.11	3.29	+2.16
Test	5.65	3.45	.10	3.55	+2.10
	5.80	4.55	.08	4.63	+1.17
After	5.80	3.71	.07	3.78	+2.02
	5.78	3.39	.05	3.44	+2.34
PIG 6-6					
Fore	5.63	2.72	0.07	2.79	+2.84
	5.76	3.16	.07	3.23	+2.53
Test	5.87	2.92	.08	3.00	+2.87
	5.89	3.52	.06	3.58	+2.31
After	5.89	2.27	.04	2.31	+3.58
PIG 6-9					
Fore	5.48	2.26	0.06	2.32	+3.16
	5.60	2.65	.06	2.71	+2.89
Test	5.70	3.22	.05	3.27	+2.43
	5.71	3.61	.03	3.64	+2.07
After	5.77	3.94	.06	4.00	+1.77
	5.75	2.92	.05	2.97	+2.78
PIG 39-30					
Fore	5.63	2.58	0.11	2.69	+2.94
	5.76	3.47	.15	3.62	+2.14
Test	5.87	3.92	.06	3.98	+1.89
	5.89	3.98	.05	4.03	+1.86
After	5.89	3.59	.07	3.66	+2 23
	5.87	3.45	.05	3.50	+2 37

TABLE 5.—*Phosphorus balances of the pigs*

[All weights in grams]

Period	Intake	Excreted in—		Total output	Balance
		Feces	Urine		
PIG 32-30					
Fore	7.91	3.63	1.16	4.79	+3.12
	8.46	4.27	1.49	5.76	+2.70
Test	8.93	5.95	1.31	7.26	+1.67
	9.01	5.65	1.27	6.92	+2.09
After	9.01	6.84	1.58	8.42	+.59
	9.12	5.91	1.52	7.43	+1.69
PIG 24-3					
Fore	7.60	3.35	1.33	4.68	+2.92
	7.56	3.92	1.31	5.23	+2.33
Test	8.02	4.41	1.46	5.87	+2.15
	8.65	5.80	1.25	7.05	+1.60
After	8.65	5.19	1.40	6.59	+2.06
	8.75	4.96	1.86	6.82	+1.93
PIG 6-6					
Fore	7.91	4.06	0.94	5.00	+2.91
	8.46	4.81	1.11	5.92	+2.54
Test	8.93	4.74	1.52	6.26	+2.67
	9.01	6.11	.76	6.87	+2.14
After	9.01	3.83	1.09	4.92	+4.09
PIG 6-9					
Fore	7.29	3.40	1.12	4.52	+2.77
	7.78	3.98	1.15	5.13	+2.65
Test	8.21	4.91	1.44	6.35	+1.86
	8.28	5.00	1.13	6.13	+2.15
After	8.52	5.90	1.28	7.18	+1.34
	8.62	4.57	1.30	5.87	+2.75
PIG 39-30					
Fore	7.91	3.99	0.90	4.89	+3.02
	8.46	4.94	1.57	6.51	+1.95
Test	8.93	5.34	1.40	6.74	+2.19
	9.01	5.88	1.35	7.23	+1.78
After	9.01	5.51	1.30	6.81	+2.20
	9.12	5.91	1.41	7.32	+1.80

TABLE 6.—*Summary of the average daily retention of nitrogen, calcium, and phosphorus by individual pigs*

NITROGEN RETENTION IN GRAMS PER DAY

Period	Pig 32-30	Pig 24-3	Pig 6-6	Pig 6-9	Pig 39-30	Average
Fore	13.87	10.27	20.03	10.35	10.99	13.10
	10.76	8.04	11.04	11.97	11.96	10.75
Test	9.85	9.87	16.99	12.15	10.51	11.87
	12.05	11.09	16.92	13.19	14.68	13.59
After	15.05	13.13	28.42	15.19	13.54	17.07
	17.27	11.52	----------	16.21	18.40	15.85

CALCIUM RETENTION IN GRAMS PER DAY

Fore	3.29	2.74	2.84	3.16	2.94	3.00
	2.93	2.16	2.53	2.89	2.14	2.53
Test	2.00	2.10	2.87	2.43	1.89	2.26
	1.89	1.17	2.31	2.07	1.86	1.86
After	1.75	2.02	3.58	1.77	2.23	2.27
	2.37	2.34	----------	2.78	2.37	2.46

PHOSPHORUS RETENTION IN GRAMS PER DAY

Fore	3.12	2.92	2.91	2.77	3.02	2.95
	2.70	2.33	2.54	2.65	1.95	2.43
Test	1.67	2.15	2.67	1.86	2.19	2.11
	2.09	1.60	2.14	2.15	1.78	1.95
After	.59	2.06	4.09	1.34	2.20	2.06
	1.69	1.93	----------	2.75	1.80	2.04

The complete balance data for nitrogen, calcium, and phosphorus are summarized in Tables 3, 4, and 5. The balances (retentions) only have been summarized in Table 6. There are no consistent differences between the data of the test period and those of the fore and after periods with respect to either nitrogen or phosphorus, and hence no suspicion that the administration of potassium iodide has affected in any way the utilization of these elements. With respect to calcium there is a suggestion of an adverse effect of potassium iodide, particularly if it is permissible to assume that such an effect may extend into the first week of the after period. The data for pigs 39–30 and 24–3 offer the clearest support for this suggestion; those for pigs 32–30 and 6–9 may also be given the same significance, though with less certainty, while the data for pig 6–6 are not clearly amenable to the same interpretation.

The experiment affords no support for Kelly's conclusion that potassium iodide administration favorably influences the metabolism of the pig.

SUMMARY

In metabolism experiments upon five pigs, in which potassium iodide was administered for 14 days in doses of 0.248 gm., and in which 14-day control periods preceded and followed this régime, there was no evidence that the utilization of either nitrogen, calcium, or phosphorus was favorably affected. There was some indication that the retention of calcium was adversely affected. No effect of the iodide administration on the rate of growth of the pigs was evident.

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VARIATIONS IN CONTENT OF SUGARS AND RELATED SUBSTANCES IN OLIVES[1]

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INTRODUCTION

In connection with a study of the composition of California olives it was found necessary to know something of the nature and amounts of the sugars and to select suitable methods for their determination. The fruit of olives of 17 varieties grown in California was therefore examined for the purpose of ascertaining (1) the amount and nature of the reducing substances in the flesh, and (2) the relation of these substances to the maturity and variety of the fruit.

REVIEW OF THE LITERATURE

Few references to the amount or nature of the sugars in olives appear in the literature. Power and Tutin,[2] studying the alcoholic extract of dried olive bark and leaves, reported the presence of sugar yielding *d*-phenylglucosazone. Bourquelot and Vintilesco [3] reported the presence in olive flesh of a phenolic compound causing Fehling's solution to indicate more than the actual amount of sugar. Scurti and Tommasi [4] in an attempt to explain the formation of oil in olives found no notable quantities of carbohydrates.

MATERIAL STUDIED

The varieties studied may be divided into three groups: (1) Varieties well established in California; Mission, Manzanillo, Sevillano, and Ascolano. These varieties are grown primarily for pickling and only the Mission is used for oil making. (2) Several varieties less widely grown—the Barouni, Columbella, Lucca, Nevadillo, Picholine, and Uvaria. (3) Varieties but recently introduced [5] into California—the Bidh el Hammam, Chemlali, Chitoni, P. I. G. 27172, Saiali Magloub, Salome, and S. P. I. 27173.

The relation between composition and maturity was studied in the Ascolano, Columbella, Manzanillo, Mission, Nevadillo, and Uvaria. In each of these varieties a series of samples was obtained from a single tree except in two instances where fruit was no longer available on the selected tree, and final samples were taken from neighboring

[1] Received for publication July 24, 1929; issued June, 1930.

[2] POWER, F. B., and TUTIN, F. THE CONSTITUENTS OF OLIVE LEAVES. Jour. Chem. Soc. [London] 93: 891–904. 1908.
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[3] BOURQUELOT, E., and VINTILESCO, J. SUR L'OLEUROPÉINE, NOUVEAU PRINCIPE DE NATURE GLUCOSIDIQUE RETIRÉ DE 'L'OLIVIER (OLEA EUROPÆA L.). Compt. Rend. Acad. Sci. [Paris] 147: 533–535. 1908.

[4] SCURTI, F., and TOMMASI, G. SULLA FORMAZIONE DEL GRASSO NEI FRUTTI OLEAGINOSI. [FORMATION OF FAT IN OLEAGINOUS FRUITS (OLIVES)]. Ann. R. Staz. Chim. Agr. Sper. Roma (II) 4: 253–286. 1910. [Abstract in Jour. Soc. Chem. Indus. 30: 1021. 1911. [Original not seen.]]

[5] The Chico, Calif., Plant Introduction Garden of the Bureau of Plant Industry, United States Department of Agriculture kindly cooperated by furnishing these varieties.

trees. In all cases an effort was made to minimize sampling errors by picking approximately half of each sample from opposite sides of the tree, and by picking all the fruit on the branches or twigs chosen at each sampling. The picking dates, at somewhat irregular intervals, ranged from October 1, 1927, to February 13, 1928, according to the maturing habits of the variety and the lateness at which sufficient fruit remained on the trees. In most instances the first picking was made when the fruit had reached about three-fourths its full size, was distinctly immature for pickling and of low oil content, and the last picking when it was rapidly falling from the trees, past its prime for oil making.

METHODS OF ANALYSIS

AMOUNTS OF SUGAR

The sugars were extracted from ground samples of the flesh by boiling with distilled water in a 500 c. c. volumetric flask for approximately 30 minutes, making up nearly to the mark, allowing to stand overnight, and then filling up to the mark. The decoction was then thoroughly shaken and strained through dry cheesecloth, and portions of the filtrate were set aside for clarification. Determinations of the amounts of sugars present were made by the picric-acid reduction method of Thomas and Dutcher.[6] Reducing sugars present both before and after hydrolysis were reported as dextrose in all cases, and regarded as simple and total sugars, respectively.

NATURE OF THE REDUCING SUBSTANCES

On a few samples dry basic lead acetate, subsequently removed by sodium oxalate, was compared as a clarifying agent with mercuric nitrate, removed by sodium bicarbonate and powdered zinc. The nature of the reducing substances present was further studied in a few samples by adding one-fifth of a commercial compressed yeast cake to a 50 c. c. portion of the filtrate prepared as previously described and brought to 37° C. The sample was thoroughly stirred to break up the yeast cake and then incubated for 20 minutes at 37° F., being shaken at 5-minute intervals. It was then clarified and examined in the usual way.

EXPERIMENTAL DATA

CLARIFICATION

Basic lead acetate was found to leave a distinctly greater reducing power in the water extract than did mercuric nitrate, as shown in Table 1. Not only were the percentages of reducing substances, both before and after hydrolysis, greater when lead was used, but the increase on hydrolysis by picric acid was also always greater. This would indicate either that the mercury removed some sucrose or that the lead failed to remove nonsugar substances, of which hydrolysis by picric acid produced or increased the reducing power. From the

[6] THOMAS, W., and DUTCHER, R. A. THE COLORIMETRIC DETERMINATION OF CARBOHYDRATES IN PLANTS BY THE PICRIC ACID REDUCTION METHOD. I. THE ESTIMATION OF REDUCING SUGARS AND SUCROSE. Jour. Amer. Chem. Soc. 46: 1662-1669. 1924.

results obtained from yeast treatment it is assumed that the lead failed to remove completely all the nonsugar substances that had reducing power either before or after hydrolysis.

TABLE 1.—*Comparison of basic lead acetate and mercuric nitrate as clarifying agents in the determination of sugars in olives*

[Reducing substances and sucrose reported as dextrose]

Variety	Date picked	Clarifying agent	Reducing substances before hydrolysis	Reducing substances after hydrolysis	Sucrose
	1927		*Per cent*	*Per cent*	*Per cent*
Ascolano	Oct. 4	Lead	5.00	6.92	1.92
		Mercury	4.62	5.42	.80
		Difference	.38	1.50	
Columbella	Oct. 1	Lead	3.46	4.34	.88
		Mercury	1.58	1.70	.12
		Difference	1.88	2.64	
Manzanillo	do	Lead	4.52	5.18	.66
		Mercury	2.04	2.28	.24
		Difference	2.48	2.90	
Mission No. 1	do	Lead	4.20	4.88	.68
		Mercury	1.88	2.16	.28
		Difference	2.32	2.72	
Mission No. 3	Oct. 4	Lead	6.40	9.20	2.80
		Mercury	4.92	5.62	.70
		Difference	1.48	3.58	
Do	Nov. 1	Lead	4.42	5.48	1.06
		Mercury	3.42	4.02	.60
		Difference	1.00	1.46	
Nevadillo No. 1	Oct. 1	Lead	4.14	5.02	.88
		Mercury	1.62	2.02	.40
		Difference	2.52	3.00	
Sevillano No. 2	Oct. 4	Lead	6.32	8.00	1.68
		Mercury	5.54	6.90	1.36
		Difference	.78	1.10	
Uvaria	Oct. 1	Lead	2.98	6.02	3.04
		Mercury	1.04	1.20	.16
		Difference	1.94	4.72	

YEAST TREATMENT

The yeast treatment was given to six olive samples, to a 0.4 per cent dextrose solution, and to distilled water, none of which had reducing power after clarification with mercuric nitrate. Three portions of one sample were clarified in three ways, namely, one with mercuric nitrate, one with basic lead acetate, and one with yeast followed by basic lead acetate. The results obtained are given in Table 2.

TABLE 2.—*Reducing substances remaining in an extract from olives after fermentation with yeast, and after clarification with different defecating agents*

[Reducing substances reported as dextrose]

Treatment	Reducing substances—	
	Before hydrolysis	After hydrolysis
	Per cent	*Per cent*
Clarified with mercuric nitrate	1.58	1.70
Clarified with basic lead acetate	3.46	4.34
Treated with yeast, then clarified with basic lead acetate	2.42	3.02

It is apparent from Tables 1 and 2 that clarification by basic lead acetate for the determination of sugars in olives by the picric-acid method appears unsatisfactory. On the other hand, reducing substances and substances having reducing power after hydrolysis that are left in the extract by mercuric nitrate appear to be limited to fermentable sugars. Mercuric nitrate was therefore used in the routine determinations of simple and total reducing substances. In the remainder of this paper the substances so determined will be considered as sugars.

RATIO OF SIMPLE TO TOTAL SUGARS

The ratios of simple to total sugars, expressed as dextrose, determined in several sets of samples representing the extremes of maturity studied are given in Table 3. The early-season mean ratio is 0.86, ranging from 0.80 to 0.89. The late-season mean ratio is 0.77, ranging from 0.67 to 0.94. Excluding the two samples not from the trees originally selected, the late-season mean ratio is 0.74, ranging from 0.71 to 0.77. The irregularity of the values obtained from samples not from the tree originally selected as shown in Table 3 and also in Table 4 indicate that the sampling error was probably large. From the ratios given it appears that the amounts of sugars principally or entirely sucrose, hydrolyzed by heating with picric acid, constitute usually from 15 to 25 per cent of the total reducing substances.

TABLE 3.—*Ratio of simple to total sugars in olives*

Variety	Date picked	Ratio
Ascolano	Oct. 4, 1927	0.85
	Jan. 3, 1928	.77
Manzanillo	Oct. 1, 1927	.89
	Feb. 13, 1928	.75
Mission No. 1	Oct. 1, 1927	.87
	Feb. 13, 1928 *	.67
Mission No. 3	Oct. 4, 1927	.88
	Nov. 1, 1927	.73
Nevadillo	Jan. 2, 1928	.71
	Oct. 1, 1927	.80
	Feb. 13, 1928 *	.94

* Fruit not from trees originally selected.

MATURITY AND SUGAR CONTENT

Only the total sugars were considered in comparing different stages of maturity and different varieties. In Table 4 are shown the per-

centages of total sugars in six varieties of olives picked on the dates indicated. In addition to the figures as determined on the fresh basis there are given the corresponding figures after calculation to the dry basis.

TABLE 4.—*Total sugars[a] in olives during ripening*

Variety	Date picked	Total sugars on—		Grams per 100 grams pits (fresh basis)
		Fresh basis	Dry basis	
		Per cent	*Per cent*	
Ascolano	Oct. 4, 1927	5.42	16.9	36.2
	Nov. 1, 1927 [b]	2.28	8.8	17.6
	Nov. 10, 1927	5.54	21.2	54.8
	Jan. 3, 1928	5.66	16.5	36.5
Columbella	Oct. 1, 1927	1.70	6.6	5.1
	Nov. 5, 1927	2.70	11.0	8.4
	Dec. 13, 1927	3.25	12.7	12.4
Manzanillo	Oct. 1, 1927	2.28	8.5	11.2
	Nov. 5, 1927	2.68	10.2	11.5
	Dec. 13, 1927	4.22	13.5	18.7
	Feb. 13, 1928	2.68	7.1	16.6
Mission No. 1	Oct. 1, 1927	2.16	6.5	5.4
	Nov. 5, 1927	2.70	8.6	10.0
	Dec. 13, 1927	3.82	10.6	16.4
	Feb. 13, 1928 [c]	3.56	6.7	6.3
Mission No. 3	Oct. 4, 1927	5.62	13.4	15.2
	Nov. 1, 1927	4.02	12.1	14.7
	Jan. 3, 1928	4.66	9.3	11.5
Nevadillo No. 1	Oct. 1, 1927	2.02	5.2	8.9
	Nov. 5, 1927	3.02	7.6	14.4
	Dec. 13, 1927	2.68	7.0	12.8
	Feb. 13, 1928 [c]	.96	1.9	4.6
Uvaria	Oct. 1, 1927	1.20	5.0	1.6
	Nov. 5, 1927	2.25	10.2	3.6
	Dec. 13, 1927	2.30	6.7	3.0

[a] Reported as dextrose.
[b] Not considered in mean, Tables 5 and 6.
[c] Not from tree originally selected.

The trend, especially in the figures on the dry basis, is for the sugar content to increase in October and November, and to decrease in December and January.

Previous investigations [7] having shown that through the range of maturity studied the average pit weight remains constant, the figures were also calculated in terms of grams of sugars per 100 g. of pits in order to indicate the absolute amounts present. Since this eliminates the distortion of percentage resulting from coincident marked changes in the oil content of the flesh, the quantitative variations are different, but the direction of the trend is the same.

Variations apparently resulting from varietal differences in maturing time have been adjusted in Figure 1 by shifting some of the curves horizontally to bring the maximum values observed to a common vertical axis. The means of the values grouped according to vertical axes were used as a basis for the smoothed curves.

VARIETY AND SUGAR CONTENT

In view of the relationship which appeared to exist between maturity and sugar content, the varieties were compared with respect to

[7] NICHOLS, P. F. RECENT INVESTIGATIONS ON OLIVE OIL. Ann. Tech. Conf. Calif. Olive Assoc. (San Francisco) Proc. 6: 73–84. 1927.

sugar content in early season, in late season, and for the entire season. In Table 5, the relative positions of the varieties are arranged according to mean sugar content on the fresh basis for the entire season. It will be noted, however, that the positions of varieties differ slightly

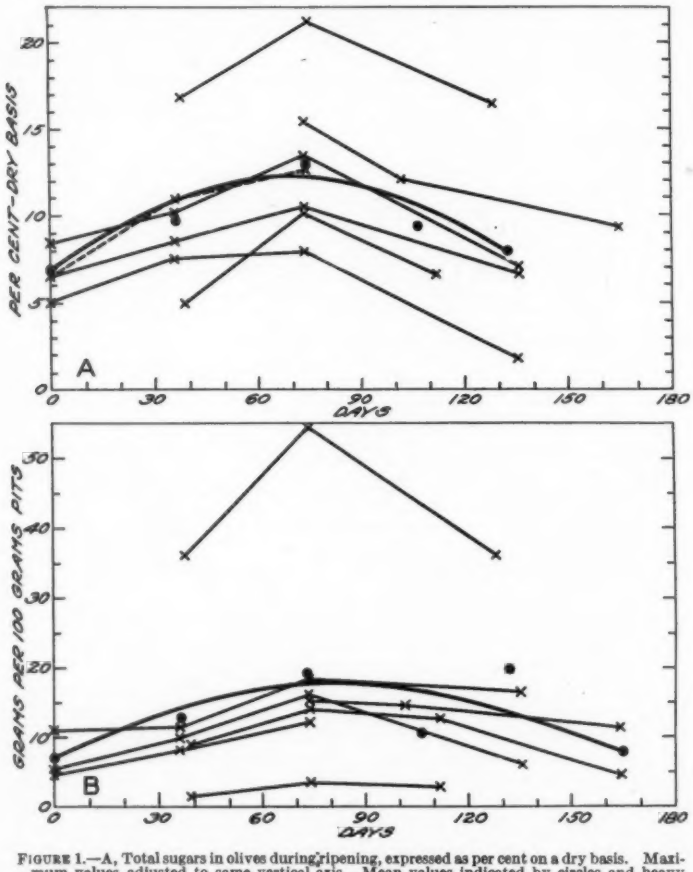

FIGURE 1.—A, Total sugars in olives during ripening, expressed as per cent on a dry basis. Maximum values adjusted to same vertical axis. Mean values indicated by circles and heavy curved line. B, Total sugars in olives during ripening, expressed as grams per hundred grams of pits. Maximum values adjusted to same vertical axis. Mean values indicated by circles and heavy curved line

from that order in the early and late seasons. The same is true in Table 6, in which the varieties are similarly arranged with respect to mean sugar content calculated on the dry basis. In almost all cases, however, the relative position remains approximately the same.

TABLE 5.—*Total sugars in olives of different varieties expressed in percentage on a fresh basis, in the early, late, and entire season*

Variety	Mean sugar content for entire season	Early season, October–November		Late season, December–February	
		Samples	Mean Sugar content	Samples	Mean Sugar content
	Per cent	*Number*	*Per cent*	*Number*	*Per cent*
Ascolano	5.54	2	5.48	1	5.66
P. I. G. 27172	4.89			2	4.89
Barouni	4.59	1	3.56	4	4.84
Sevillano	4.09	3	4.87	2	2.92
Mission	3.68	4	3.62	4	3.74
Chitoni	3.57	1	3.22	2	3.75
Manzanillo	2.96	2	2.48	2	3.45
Chemlali	2.72	1	2.15	2	3.00
Columbella	2.55	2	2.20	1	3.25
Nevadillo	2.55	3	2.67	4	2.46
Bidh el Hammam	2.39	1	2.04	2	2.56
Saiali Magloub	2.06	1	1.48	2	2.35
Uvaria	1.92	2	1.72	1	2.30
S. P. I. 27173	1.54	1	1.54		
Lucca	1.52	1	1.52		
Picholine	1.16			1	1.16
Salome	1.14	1	1.14		

TABLE 6.—*Total sugars in olives of different varieties expressed in percentage on a dry basis, in the early, late, and entire season*

Variety	Mean sugar content for entire season	Early season, October–November		Late season, December–February	
		Samples	Mean Sugar content	Samples	Mean Sugar content
	Per cent	*Number*	*Per cent*	*Number*	*Per cent*
Ascolano	18.2	2	19.0	1	16.5
Sevillano	16.8	3	21.1	2	10.4
Barouni	13.3	1	15.2	4	12.8
P. I. G. 27172	10.6			2	10.6
Columbella	10.1	2	8.8	1	12.7
Manzanillo	9.8	2	9.4	2	10.3
Mission	9.5	4	10.6	4	8.4
Chitoni	9.0	1	8.2	2	9.0
Uvaria	7.3	2	7.6	1	6.7
Bidh el Hammam	7.2	1	6.6	2	7.4
Chemlali	7.0	1	6.3	2	7.4
Nevadillo	6.5	3	10.2	4	6.2
Saiali Magloub	6.0	1	4.7	2	6.6
S. P. I. 27173	5.3	1	5.3		
Lucca	5.3	1	5.3		
Salome	4.4	1	4.4		
Picholine	3.0			1	3.0

In view of the wide and inconsistent variations shown in Table 4 these means can not be used for close comparisons of varieties. In a relative way, however, the varieties may be divided into three groups with respect to sugar content: Ascolano, P. I. G. 27172, Barouni, and Sevillano, which were high; Mission, Chitoni, Manzanillo, Chemlali, Columbella, Nevadillo, Bidh el Hammam, Saiali Magloub, and Uvaria, which were medium; and S. P. I. 27173, Lucca, Picholine, and Salome, which were low.

SUMMARY

Basic lead acetate was found to be unsatisfactory as a clarifying agent, and to give too high results in the picric acid determination of reducing sugars and sucrose in the water extract of olive flesh.

Reducing substances before and after hydrolysis, remaining in the water extract after clarification by mercuric nitrate were found to be removable by a short treatment with yeast and are believed to be sugars.

The early-season mean ratio of simple to total sugars, expressed as dextrose, usually declined as the season progressed.

The total sugar percentage on the dry basis and in absolute amounts was found to increase in early season and to decrease in late season.

Of the varieties studied Ascolano, P. I. G. 27172, Barouni, and Sevillano were high in sugar content; S. P. I. 27173, Lucca, Picholine, and Salome were low; and the Mission, Chitoni, Manzanillo, Chemlali, Columbella, Nevadillo, Bidh el Hammam, Saiali Magloub, and Uvaria were intermediate.

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